Intrada WP-RP

High Resolution Protein Separation

- *Improved Protein Recovery for Polymer Separation*

  Reverse-column tailored with a 30nm pore size

  Optimal for the separation of proteins and other large molecules up to 300,000 Da

- *Low Carryover*

  Unique packing reduces carryover

- *Superior Resolution Column with 3µm Particles*

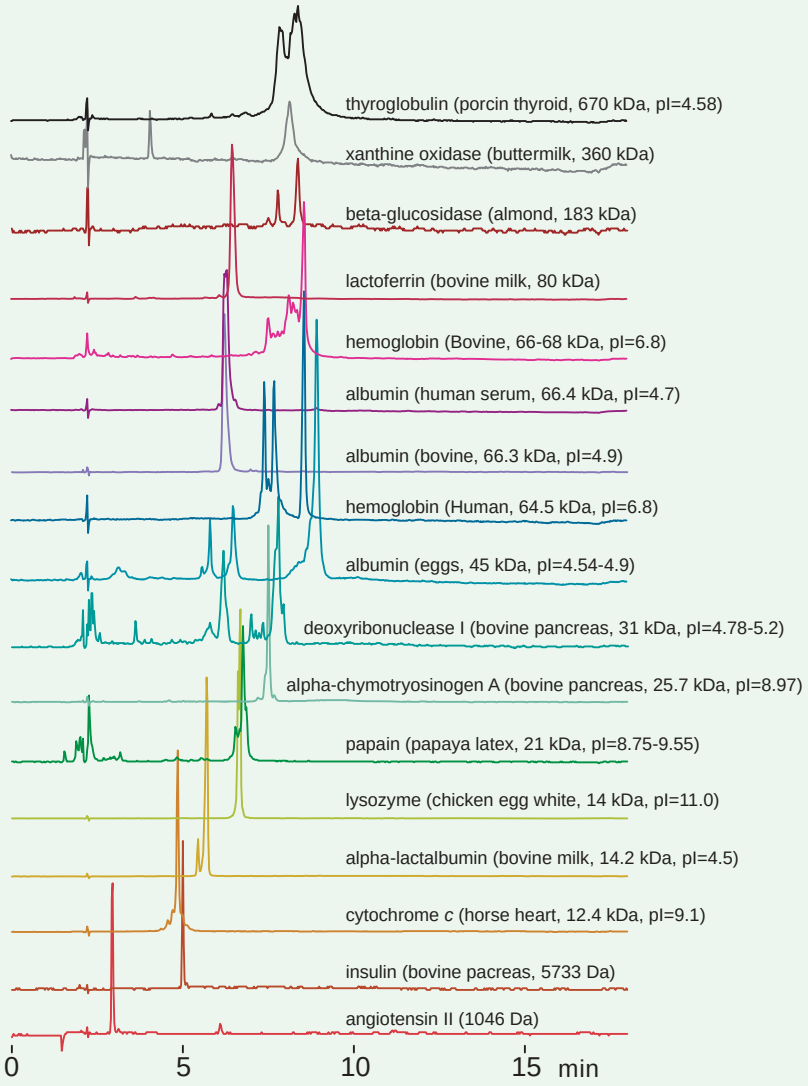
  High Resolution 3µm Silica is used

  Radically improved column efficiency compared with conventional 5µm columns

- *Optimal Surface Polarity for Faster Polymer Elution*

  Uses a newly developed reverse phase ligand

  Highly hydrophobic polymer elution made possible by optimal surface polarity



Intrada WP-RP, 150 x 4.6 mm, 1 mL/min, 37 degC, 280 nm
A: water /TFA=100 /0.1, B: ACN /TFA=100 /0.1, 20-70%B (0-15min)

The chromatograms above show the relationship between molecular weight and retention. For the reverse phase separation of large proteins (greater than 10,000 Da), a wide pore (300A) column should be used. Intrada WP-RP (300A) is an excellent column of choice for the reverse phase separation of large, highly hydrophobic polymers and proteins (up to 300,000 Da).

Key specifications: 3µm particle size, 30nm pore size, ligand for reverse phase, polymeric endcapping

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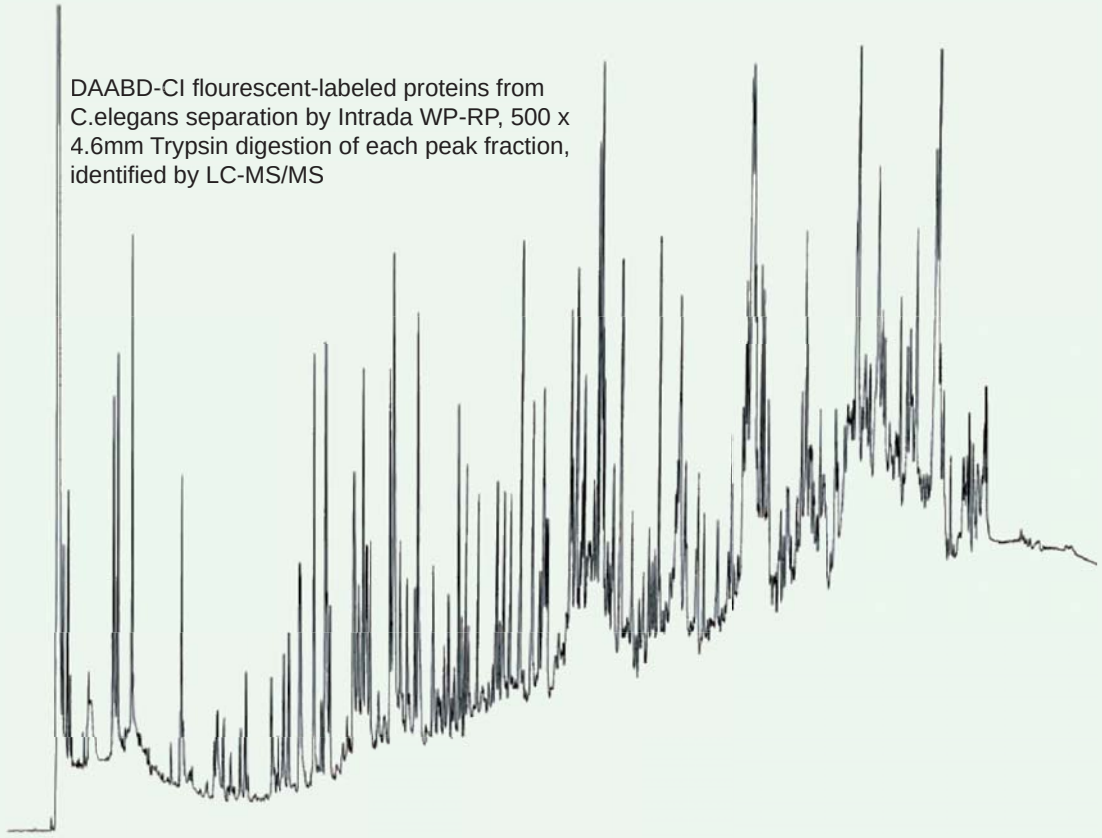
215-665-8902

High Resolution Separation of 111 Proteins (9-225 kDa)

An improved method of proteomics study in C. elegans



Peak no. Wormpep ID Protein MW (kDa)

1 CE05747 Ribosomal protein S23 15.8
2 CE16792 ATPase inhibitor 12.0
3 CE00450 60S ribosomal protein L35 14.2
4 CE08526 Ribosomal protein L13 23.7
5 CE35965 Hypothetical protein F15E11.12 35.8
6 CE30781 Ribosomal protein YL39 16.1
7 CE06313 60S ribosomal protein L28 13.7
8 CE27691 Hypothetical protein C16A3.8 17.3
9 CE04362 60S ribosomal protein L35A 13.8
10 CE05860 Ribosomal protein S11 17.7
11 CE29008 Ribonucleoprotein 33.4
12 CE26911 60S ribosomal protein L34 like 12.7
13 CE16012 40S ribosomal protein S26 13.2
14 CE16999 Hypothetical protein F15E11.1 17.4
15 CE04102 Ribosomal protein L22 14.9
16 CE30023 Ribosomal protein L22/L17 21.5
17 CE19677 rpl-14 15.3
18 CE00744 Ribosomal protein ML16 24.3
19 CE05598 60S ribosomal protein L3 45.6
20 CE16308 RNA recognition motif 9.3
21 CE27691 Hypothetical protein C16A3.8 (same as 8) 17.3
22 CE24592 40S ribosomal protein S6 28.1
23 CE14956 Ribosomal protein S13 17.8
24 CE00664 rps-1 28.9
25 CE01543 Ribosomal protein L10 24.7
26 CE26774 Ribosomal protein L27 16.2
27 CE04805 Major sperm protein msp-32 21.3
28 CE16650 Eukaryotic ribosomal protein L18 21.0
29 CE20707 Elongation factor 156.1

Peak no. Wormpep ID Protein MW (kDa)

30 CE17730 cyp-5 22.4
31 CE21392 rpl-20 20.9
32 CE10598 y-Box protein 2 29.4
33 CE02255 60S ribosomal protein L5 33.4
34 CE00778 rpl-23 14.9
35 CE01810 Ribosomal protein S3 27.3
36 CE27398 Ribosomal protein L7Ae/L30e/S12e/Gadd45 30.2
37 CE30401 Ribosomal protein L7Ae/L30e/S12e/Gadd45 27.9
38 CE16308 RNA recognition motif (same as 20) 9.4
39 CE12918 40S ribosomal protein S16 16.3
40 CE08720 Acyl-coA-binding protein 9.4
41 CE21562 Calreticulin 45.6
42 CE17986 Ribosomal protein L11 17.8
43 CE01030 L13P family ribosomal protein 23.0
44 CE00821 Ribosomal protein S14 16.2
45 CE08526 Ribosomal protein L13 (same as 4) 23.0
46 CE17730 cyp-6 21.9
47 CE34269 Myosin light chain 1 18.6
48 CE20371 cyp-7 18.4
49 CE04038 Disorganized muscle protein 1 35.5
50 CE25224 Hypothetical protein F15E11.13 17.2
51 CE00994 Heat shock hsp20 proteins 17.8
52 CE06652 Glutamate dehydrogenase 58.6
53 CE02420 cyp-2 18.4
54 CE20371 cyp-7 (same as 48) 18.4
55 CE20413 rpl-30 12.2
56 CE01236 Myosin light chain 3 17.1
57 CE30433 60S acidic ribosomal protein p2 10.8
58 CE09945 40S ribosomal protein S15 17.2

Peak no. Wormpep ID Protein MW (kDa)

59 CE19095 Hypothetical protein Y38H6C.1 9.9
60 CE05849 Ribosomal protein S9 21.9
61 CE05165 Histone H2B 15.6
62 CE07669 Ribosomal protein L1 38.6
63 CE29558 Hypothetical protein C04C3.2 29.9
64 CE29443 Channel protein 33.0
65 CE25552 rpl-1 24.1
66 CE09349 Myosin heavy chain B 225.0
67 CE11024 rpl-7 28.1
68 CE28782 Tropomyosin, LEVamisole resistant LEV-11 33.0
69 CE06253 Myosin heavy chain 224.6
70 CE24278 Ribosomal protein S4E and KOW 29.0
71 CE22694 Ribosomal protein rpa-2 10.9
72 CE09650 Nucleoside diphosphate kinase 17.1
73 CE30646 Fructose-1,6-bisphosphate aldolase class-I, CE2 isozyme 38.8
74 CE01270 Elongation factor 1-ƒ¿ 50.6
75 CE09512 Malate dehydrogenase 35.1
76 CE09719 ATPase 25.6
77 CE09512 Malate dehydrogenase (same as 75) 35.1
78 CE06577 40S ribosomal protein S7 30.7
79 CE03684 Enolase 46.6
80 CE00134 far-2 20.0
81 CE19610 Phosphoenolpyruvate carboxykinase 73.4
82 CE06577 40S ribosomal protein S7 (same as 78) 22.0
83 CE08110 hsp-70 70.6
84 CE18826 Hypothetical protein H28O16.1a 57.8
85 CE09115 Mitochondrial 40S ribosomal protein S5 23.1
86 CE28594 vit-6 193.3
87 CE32364 Hypothetical protein F09E5.2 65.1

Peak no. Wormpep ID Protein MW (kDa)

88 CE09655 Deoxyribonuclease 33.8
89 CE28594 vit-6 (same as 86) 193.1
90 n.r. Actin 54.7
91 CE03921 vit-5 186.3
92 CE04589 Arginine kinase 42.0
93 n.r. Ribosomal protein P1 homolog 11.3
94 CE16463 Actin 41.8
95 CE00513 Citrate synthase 51.5
96 CE05854 Hypothetical protein F40F9.5 33.2
97 CE01431 Annexin family protein 1 35.7
98 CE13150 act-2 41.8
99 CE03921 vit-5 (same as 91) 186.3
100 CE09682 HSP-1 heat shock 70kd protein 69.7
101 CE19186 Ribosomal protein L7Ae 14.0
102 CE12898 ADP/ATP translocase 33.0
103 n.r. Translation elongation factor eEF-2 94.7
104 CE20900 vit-3 186.4
105 n.r. Chain A, crystal structure of C. elegans Mg-Atp 41.6 actin complexed with human gelsolin segment 1
106 CE29950 ATP synthase beta chain (atp-2) 57.5
107 CE13148 Actin 41.8
108 CE00548 Elongation factor 1 22.7
109 CE06200 14-3-3-like protein 28.1
110 CE07648 Cystathionine r-lyase 43.1
111 CE32385 Glutathione S-transferase, C-terminal and elongation factor 1, r-chain 42

Intrada WP-RP, 500x4.6mm, A) water/ACN/TFA=90/10/0.1, B) water/ACN/TFA=30/70/0.1, 0-20%B(0-20min), 20-60%B(20-180min), 0.5mL/min, Ex.387nm, Em.508nm, 30uL

Courtesy of Prof. Imai, Musashino Univ. *M.Masuda, H.Saimaru, N.Takamura and K.Imai, Biomed. Chromatogr., 19, 556-560 (2005)*

The above chromatogram used a new proteomic analytical method called “flourescent labeled protein method” with Intrada WP-RP. The 3um particle, 500mm column provides the ability to separate large numbers of proteins.

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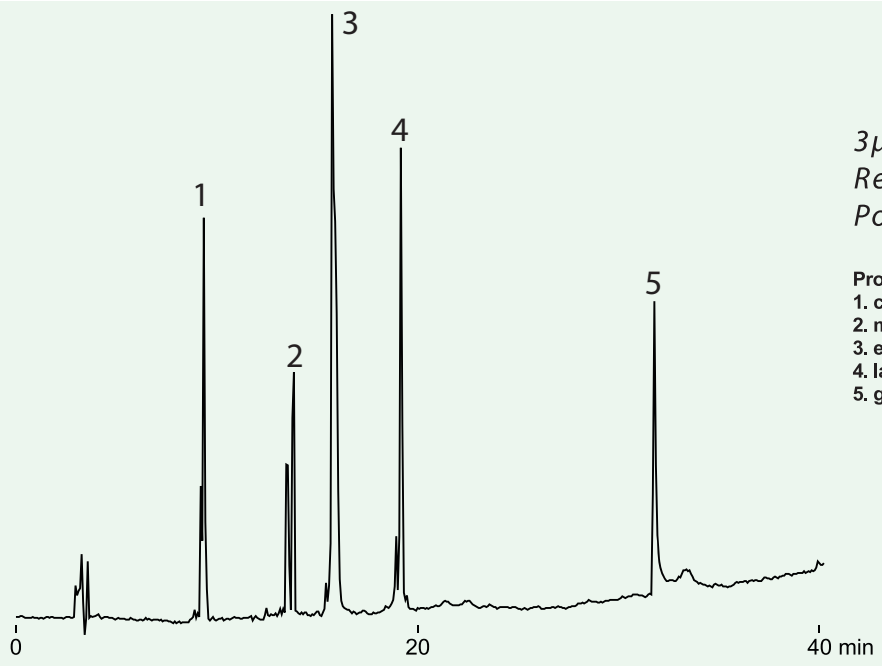
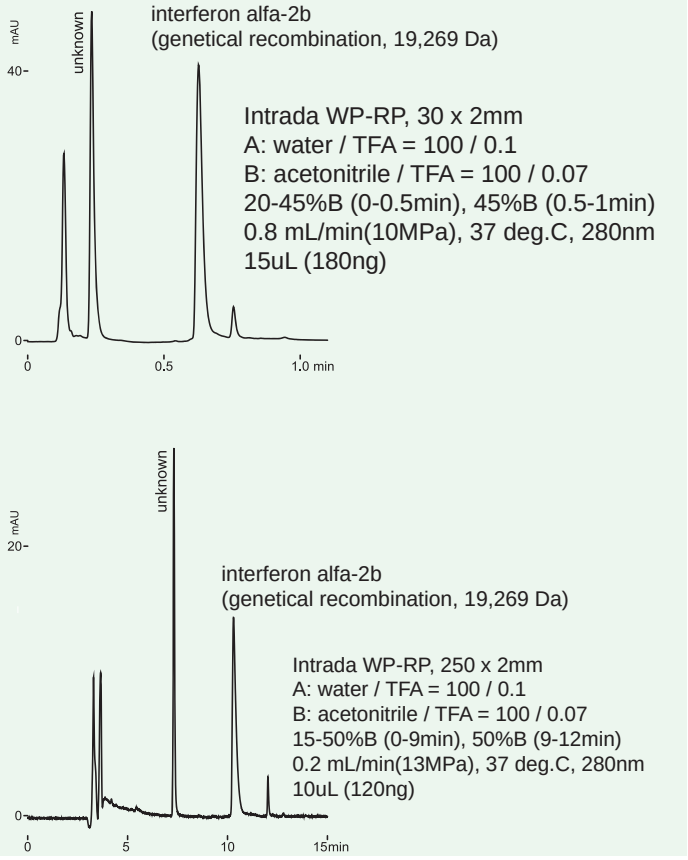
Excellent Peak Shape



3µm, 30mm, Pure Silica
Reversed Phase
Polymeric Endcapping

Proteins	M.W. (kDa)
1. cytochrome c (horse heart)	12.4
2. myokinase (yeast)	32
3. enolase (yeast)	67
4. lactate dehydrogenase (pig heart)	142
5. glutamate dehydrogenase (yeast)	290

Effective Interferon Retention

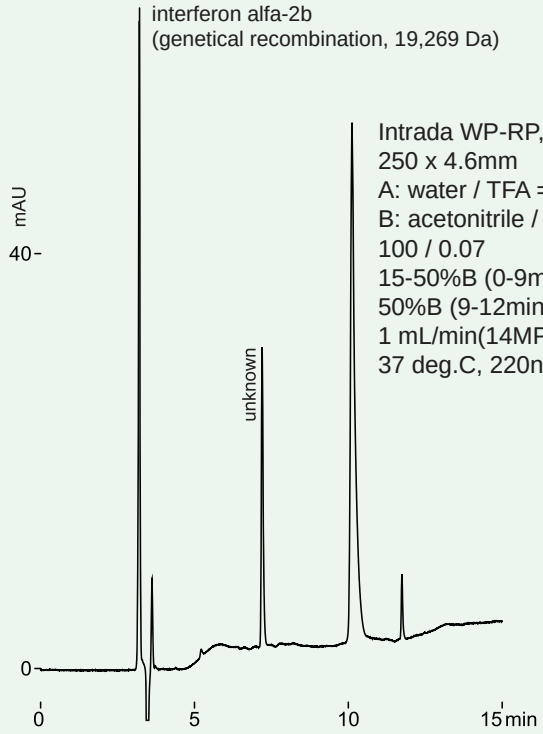


interferon alfa-2b
(genetical recombination, 19,269 Da)

Intrada WP-RP, 30 x 2mm
A: water / TFA = 100 / 0.1
B: acetonitrile / TFA = 100 / 0.07
20-45%B (0-0.5min), 45%B (0.5-1min)
0.8 mL/min(10MPa), 37 deg.C, 280nm
15uL (180ng)

interferon alfa-2b
(genetical recombination, 19,269 Da)

Intrada WP-RP, 250 x 2mm
A: water / TFA = 100 / 0.1
B: acetonitrile / TFA = 100 / 0.07
15-50%B (0-9min), 50%B (9-12min)
0.2 mL/min(13MPa), 37 deg.C, 280nm
10uL (120ng)



interferon alfa-2b
(genetical recombination, 19,269 Da)

Intrada WP-RP,
250 x 4.6mm
A: water / TFA = 100 / 0.1
B: acetonitrile / TFA =
100 / 0.07
15-50%B (0-9min),
50%B (9-12min)
1 mL/min(14MPa),
37 deg.C, 220nm

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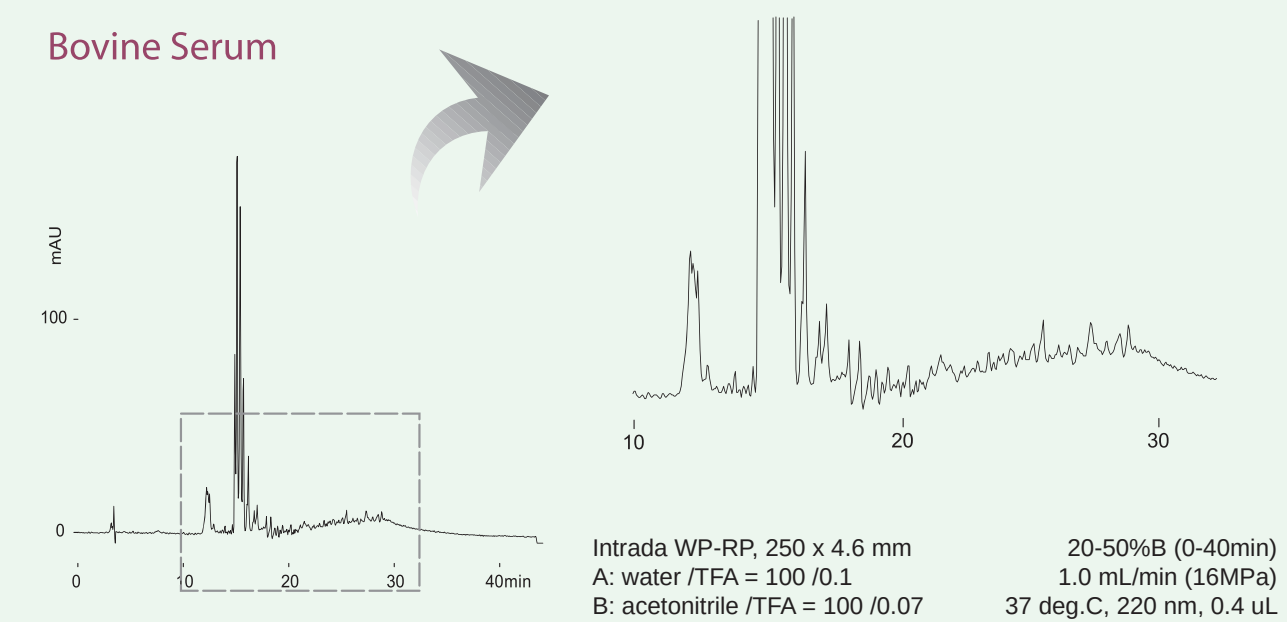
Intrada WP-RP: Exceptional Protein Separation



Ordering Information for Intrada WP-RP 3µm

3µm Column, Pressure limits of up to: 20MPa, 250 bar, 3,000 psi							
	Internal Diameter						
Column Length	1.0 mm	1.5 mm	2.0 mm	3.0 mm	4.6 mm	6.0 mm	10.0 mm
10			WPR20	WPR30	WPR00		
20			WPR29	WPR39	WPR09		
30	WPR11	WPR71	WPR21	WPR31	WPR01	WPR61	WPRP1
50	WPR12	WPR72	WPR22	WPR32	WPR02	WPR62	WPRP2
75	WPR13	WPR73	WPR23	WPR33	WPR03	WPR63	WPRP3
100	WPR14	WPR74	WPR24	WPR34	WPR04	WPR64	WPRP4
150	WPR15	WPR75	WPR25	WPR35	WPR05	WPR65	WPRP5
250	WPR16	WPR76	WPR26	WPR36	WPR06	WPR66	WPRP6
500					WPR07		

Guard Column System for Intrada WP-RP							
	Internal Diameter						
	1.0 mm	1.5 mm	2.0 mm	3.0 mm	4.6 mm	6.0 mm	10.0 mm
Guard Holder	GCH01S	GCH01S	GCH01S	GCH01S	GCH01S	GCH01S	GCH02M
Guard Cartridge (Set of 3)	GCWPRC	GCWPRC	GCWPRS	GCWPRS	GCWPRS	GCWPRS	GCWPRM

All of our stationary phases can also be made in the following additional internal diameters:
Nano: 0.05mm, 0.075mm Capillary: 0.1mm, 0.3mm, 0.5mm

Four Easy Ways To Order:
1. Call us at (215) 665-8902
2. Order by fax (501) 646-3497
3. Through VWR (vendor code 8070779) or Fisher (vendor code VN101253)
4. Via www.imtaktusa.com with any major credit card

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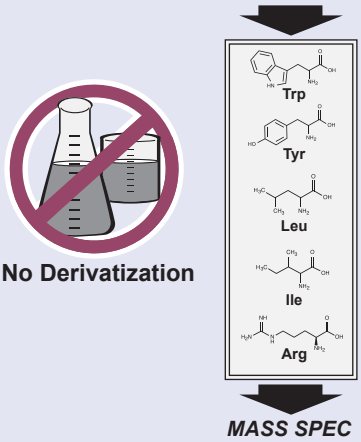
Imtakt USA

Imtakt USA Intrada Amino Acid

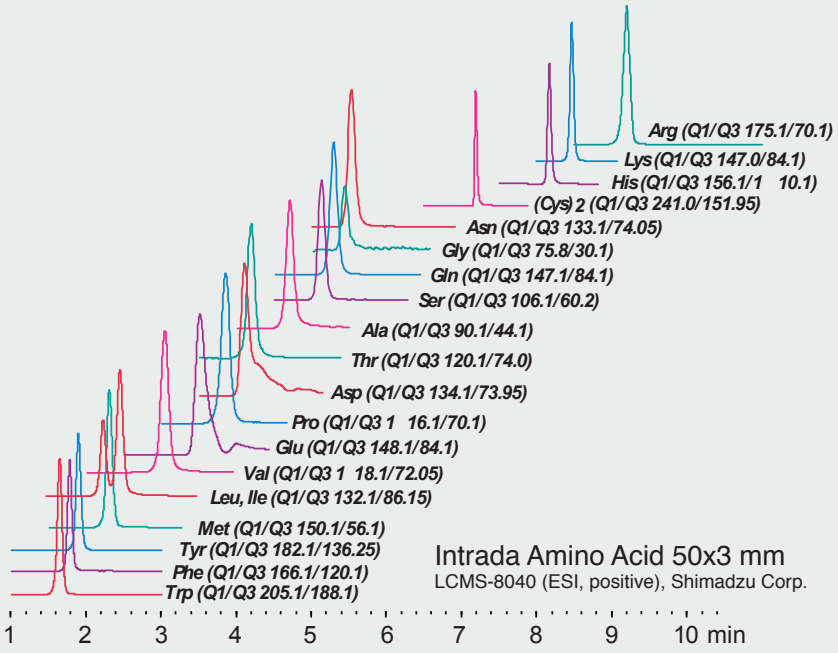
Amino Acids Separation Column for LC-MS

The world's first specialty column for intact amino acid analysis via LC-MS

- *LC-MS analysis of amino acids*
- *Amino Acid Analysis - No derivatization required*
- *Ability to separate isobaric amino acids such as Leu and Ile*
- *High-throughput (< 1 minute) analysis for selected amino acids*
- *5-10 minutes for protein amino acids analysis*
- *Pure spherical silica / 3um particles / unique stationary phase designed for amino acid*



Separation of amino acids under 10 minutes using LC-MS/MS



Standard Amino acid mixture
100 nmol/mL

A: ACN / THF / 25 mM Ammonium formate / Formic acid = 9 / 75 / 16 / 0.3 (v/v/v/v)
B: ACN / 100 mM Ammonium Formate = 20 / 80 (v/v)

Gradient Conditions:
0%B (0-3 min)
0-17%B (3-6.5 min)
100%B (6.5-10 min)
0.6 mL/min, 40 °C, 1 µL (0.1N HCl)
ESI, positive

Imtakt has developed a novel column for the analysis of amino acids with LC-MS systems. The Intrada Amino Acid column achieves high-throughput analysis without tedious pre- or post-labeling methods. Optimization of analytical run times and resolution is accomplished by varying column dimensions. Separation of leucine and isoleucine isomers, GABA isomers, and dipeptide analysis is now possible without derivatization.

- *Separate free amino acids in mixtures*
- *Study protein amino acid composition*
- *Isolate amino acid bio-markers*

Imtakt USA (info@imtaktusa.com) *North America*
Imtakt Corp./JAPAN (info@imtakt.com) *Other Countries*

LC-MS analysis for 55 amino acids in 10 minutes

- New stationary phase designed specifically for optimal amino acid and dipeptide separation
- No pre- or post-labeling methods required
- High throughput analysis via LC-MS
- Separation of isobaric amino acids on LC-MS systems is finally possible

55 Amino Acids (standard samples)

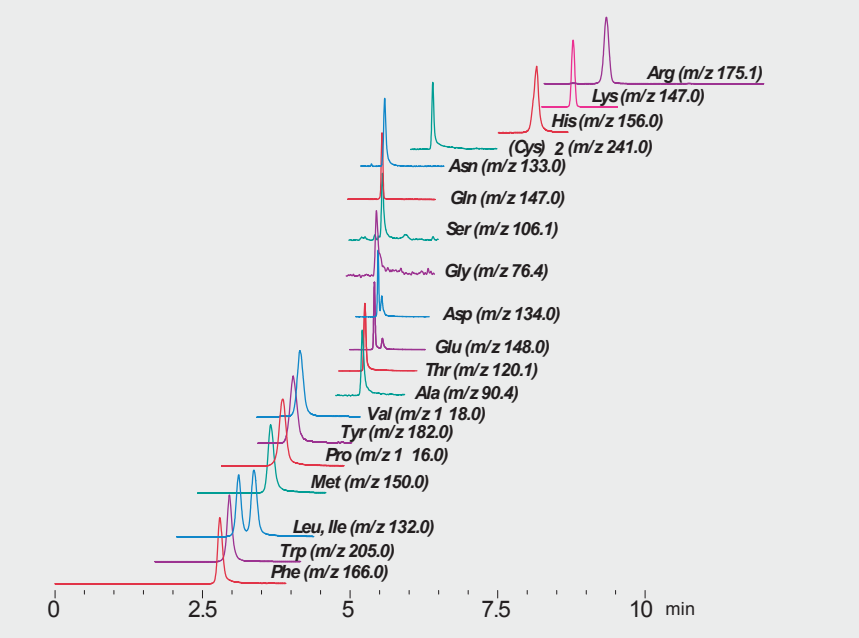
Intrada Amino Acid, 50 x 3 mm
A: ACN / THF / 25mM Ammonium formate / Formic acid = 9 / 75 / 16 / 0.3
B: ACN / 100mM Ammonium formate = 20 / 80

Gradient Conditions:
0 %B (0-2.5min)
0-17 %B (2.5-6.5 min)
100 %B (6.5-10 min)
0.6 mL/min (6MPa), 35 °C , 5 μL (1μmol/mL)
ESI (SIM, positive)

Various column dimensions enhance scalability and flexibility

- Mobile phase composition and gradient method can be optimized for sensitivity requirements and run times.
- Use of a shorter column allows for one minute analysis of non-isomer amino acids.

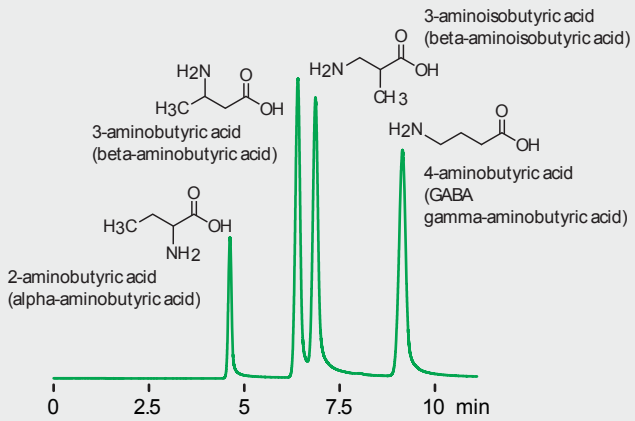
Example of simple elution method



Intrada Amino
Acid, 50 x 3 mm
A: ACN / Formic acid = 100 / 0.1
B: 100mM Ammonium formate

Gradient Conditions:
14 %B (0-3min)
14-100 %B (3-10 min)
14 %B (10-12 min)
0.6 mL/min, 35 °C, 5 µL
ESI, positive

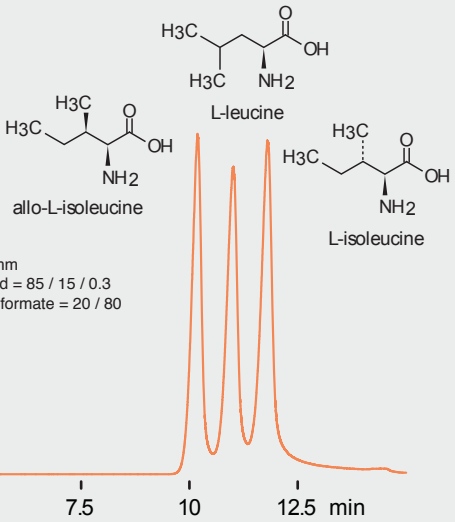
Separation of GABA (103Da) isomers



Intrada Amino Acid, 100 x 3 mm
A: ACN / Formic acid = 100 / 0.3
B: 100mM Ammonium formate

Gradient Conditions:
25-30 %B (0-12 min),
100 %B (12-15min)
0.4 mL/min (4MPa), 37 °C, 5 µL (0.1N HCl)
ESI (positive, m/z 104.1)

Separation of Leucine (131Da) isomers

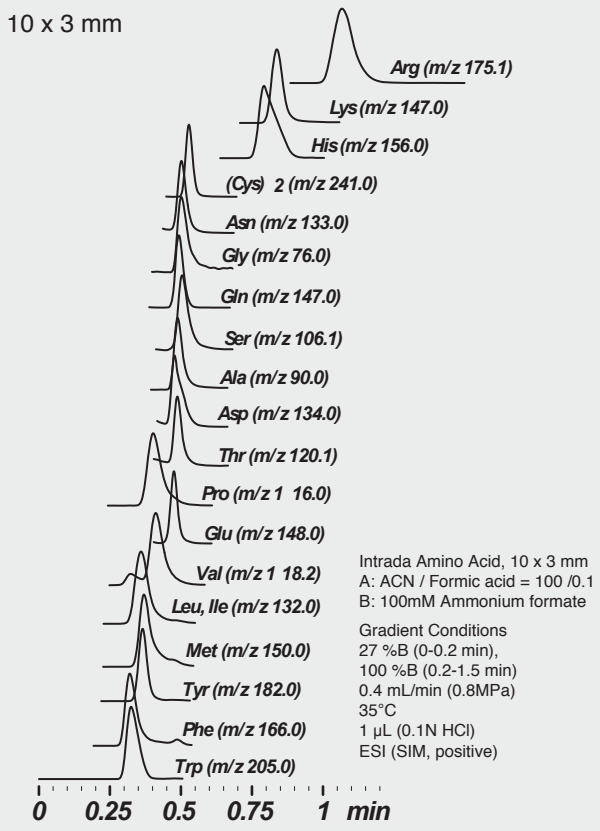


Intrada Amino Acid, 150 x 3 mm
A: MeOH / Water / Formic acid = 85 / 15 / 0.3
B: ACN / 100mM Ammonium formate = 20 / 80

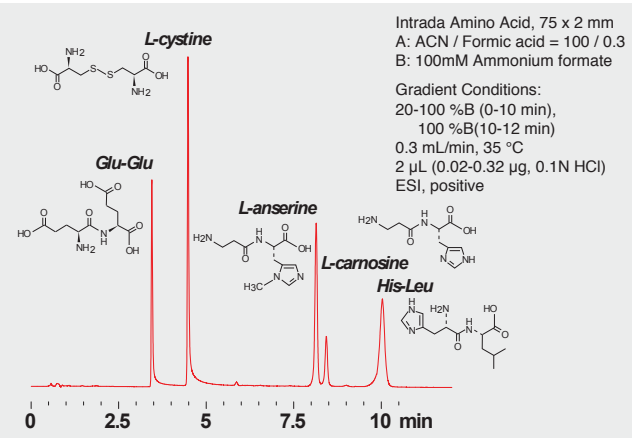
Gradient Conditions:
12-13 %B (0-12 min)
100 %B (12-15 min)
0.4 mL/min (11 MPa)
35 °C, 5 µL (0.1N HCl)
ESI (positive, m/z 132.0)

Intrada Amino Acid columns separate amino acid isomers quickly by using an optimized column length, as in the example of aminobutyric acid isomers (103Da) and leucine isomers (131Da) using 100-150mm length columns.

High-throughput analysis of standard amino acids



Dipeptide analysis



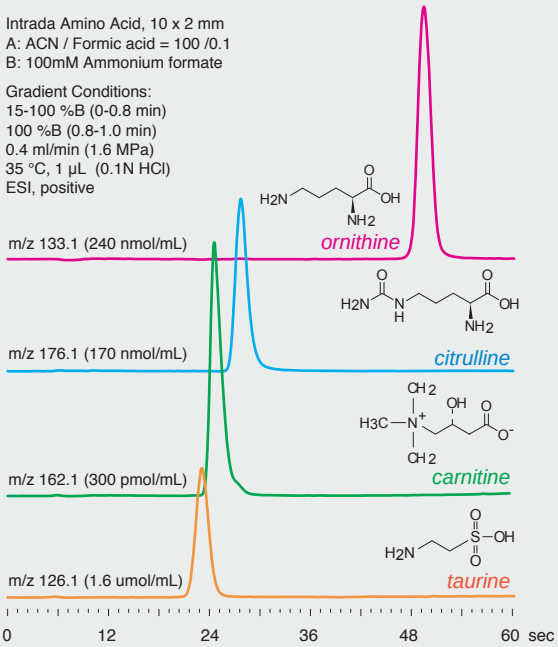
Intrada Amino Acid is an excellent choice for the analysis of polar dipeptides, which are notoriously difficult to retain and separate in conventional HPLC.

Product Information

Column I.D.	Column Length (depends on I.D.)
3mm, 2mm, 1mm 0.5mm - 0.075mm	10mm, 20mm, 30mm 50mm, 75mm, 100mm 150mm, 250mm

Guard column system is not available for this product.

One-minute analysis of related compounds



Above: One minute ultra high-throughput analysis can successfully be performed on a 10mm length column.

Intrada Amino Acid high-throughput columns provide amazing speed, selectivity, and convenience. The next generation amino acid analysis method for clinical amino acid biomarkers, fermented materials, botanical amino acids is here.

Column Recommendations

- The Intrada Amino Acid column should be used only with LC-MS systems to achieve adequate peak identification.
- This product is not recommended for applications involving UV or ELSD instruments.
- Detection sensitivity is highly dependent upon MS instrument performance. LC-MS instruments should be carefully chosen to yield adequately sensitive data.
- Analysis of longer chain peptides that require high ionic strength mobile phases should use the Scherzo SS-C18 multi-mode ODS column.
- Please refer to the instruction packet for sample preparation procedures.

Imtakt USA

An Innovative Size Exclusion Column Compatible with GFC/GPC

World's first silica-diol SEC column designed to work with LC and LC-MS compatible volatile salts

Intrada *SEC*

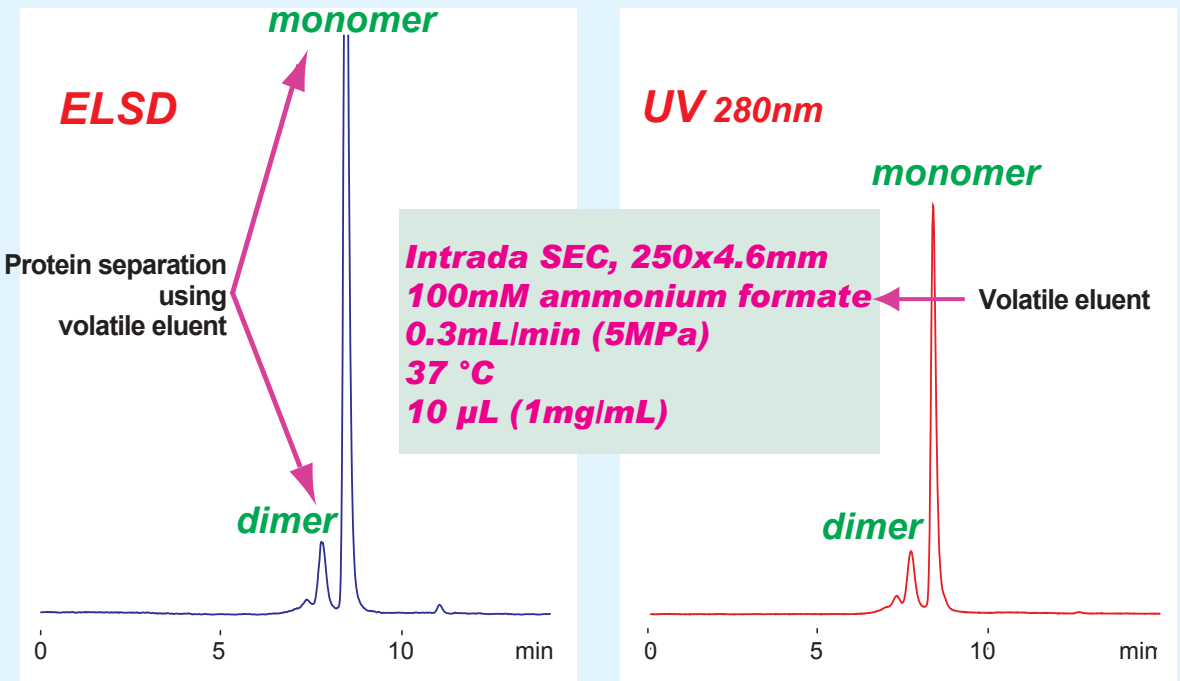
Compatible with hydrophilic polymers like proteins (GFC)
Compatible with hydrophobic polymers like polystyrene (GPC)
Uses volatile solvents as standard eluents
Extended lifetime under pH 1-8

Spherical porous silica / 3um particle / 30nm pore / Diol phase / pH 1-8 / MW up to 1MDa

Does not require NaCl for protein separation

BSA (Bovine Serum Albumin)

Monomer 66 kDa
Dimer 132 kDa



Intrada SEC is a wide pore (300A), fully porous silica material with a bonded diol substitution stationary phase. This innovative technology overcomes some traditional problems with diol-silica columns, such as the need for use of high concentrations of non-volatile salts in the eluent and poor lifetime due to undesired residual silanols effect. The next generation Intrada SEC column allows both aqueous durability like a polymer-based SEC column and the mechanical strength of a silica-based SEC column. This column can be used with volatile salts, such as 100mM ammonium formate, as the eluent for aqueous polymer analytes, making it compatible with ELSD or MS detection.

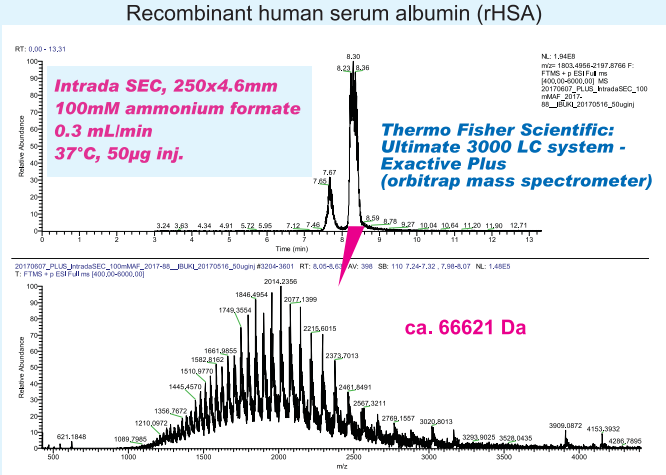
Another benefit of the technology behind the Intrada SEC column is that it can be used for both aqueous GFC (proteins, polysaccharides and nucleic acids etc.) and non-aqueous GPC (polystylene, PVAc etc.), making it a real unified "SEC" column.

Ability to use volatile salt eluents may change SEC history

Traditionally, it has been required to use 100mM phosphate buffer + 300mM NaCl as an eluent for diol-silica SEC columns due to an undesired ionic effect, caused by exposed residual surface silanols. But use of high concentrations of inorganic salt is too harsh for use with most LC systems and also their non-volatile nature makes them incompatible for use with an MS detector. The innovative Intrada SEC column with a novel diol substitution technology allows for the use of 100mM ammonium formate instead, which is a volatile eluent that is compatible with MS and ELSD detection. This truly revolutionary design may change the history of SEC.

SEC-MS: Recombinant HSA — Intrada *SEC*

Recombinant human serum albumin (rHSA)



Courtesy of Dr. Keiko KUWATA, Nagoya University, JAPAN

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Intrada SEC may be the best SEC column for use on MS detectors, due to its use of volatile eluents.

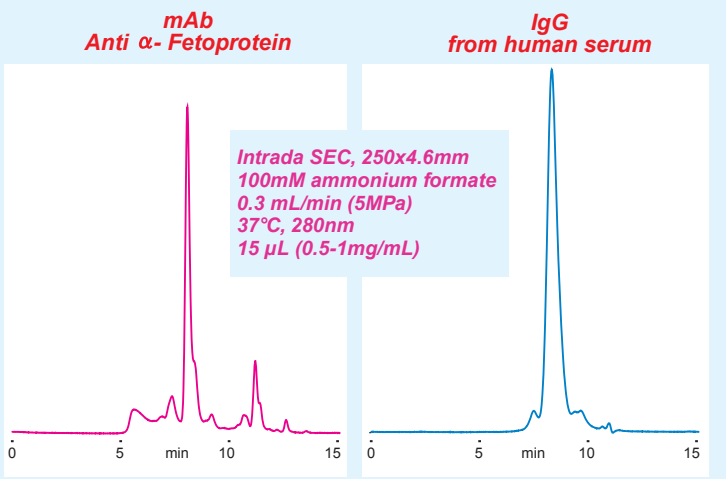
The figure to the left shows recombinant human serum albumin (rHSA) monomer and dimer separation on LC-MS. The molecular weight of the monomer was directly analyzed as 66621Da, which is more accurate than the traditional caliburation curve method, which is based on the hypothesis that all of the proteins are globular shape.

We feel this shows that SEC-MS using Intrada SEC column will likely be an improved method for MW determination in the future.

Determination of mAb purity is critical in antibody drug science. To further support this work, Intrada SEC columns allow the separation of not only monomers but also oligomers and even fragments, as the figure to the right shows.
For purification needs for products such as biopolymers, antibodies or enzymes, full preparative column dimensions are available (10 - 20 mm ID). Further adding to the convenience of using Intrada SEC for purification work, this unique material is designed to work conveniently with HPLC instruments, due to its ability to utilize volatile buffer eluents.

Polymer quantification is also possible with Intrada SEC columns, as is shown below, where accurate quantification of a 660 kDa large protein is performed. Improved separation performance and accuracy can also be achieved by using our u-shaped multi-column connections, which enable up to four columns to be connected in series.

mAb, IgG — Intrada *SEC*



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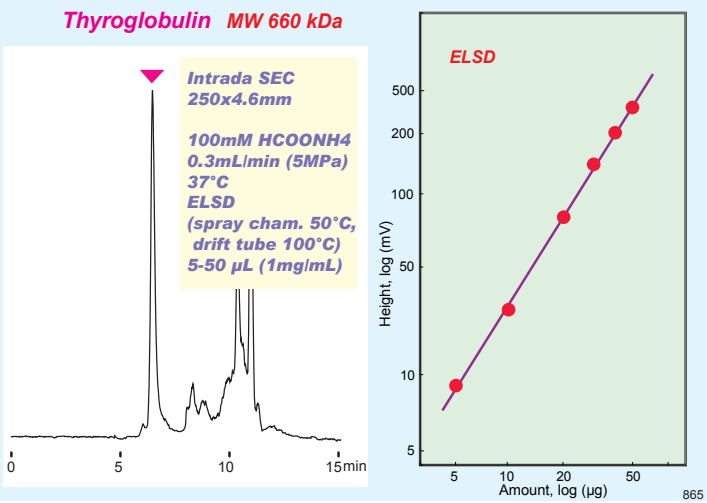
Quantification — Intrada *SEC*

Thyroglobulin MW 660 kDa



865

Product Information

Intrada *SEC*

Separation Column

250 x 4.6 mm : WSE06
250 x 10 mm : WSEP6
250 x 20 mm : WSEQ6

Guard Cartridge

4.6mmI.D. : GCWSES
10-20mmI.D. : GCWSEM

Guard Holder

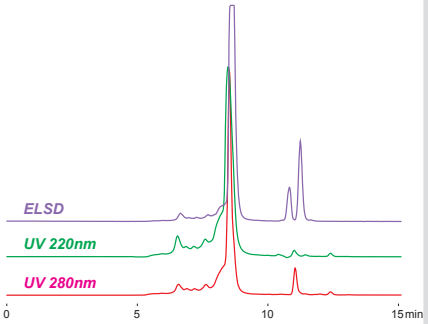
4.6mmI.D. : GCH11S
10-20mmI.D. : GCH12M

Versatile SEC column for peptides, proteins, bio- and synthetic polymers

Peptides, Proteins / Bio-Polymers

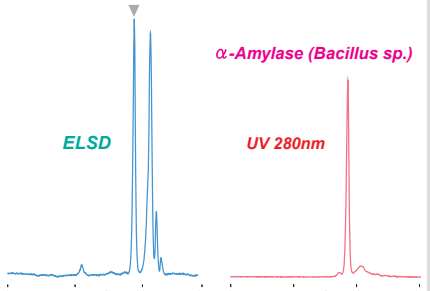
Nearly all bio-polymers are compatible with the use of volatile 100mM ammonium formate as the eluent solution, as shown in the examples below.

Human Serum



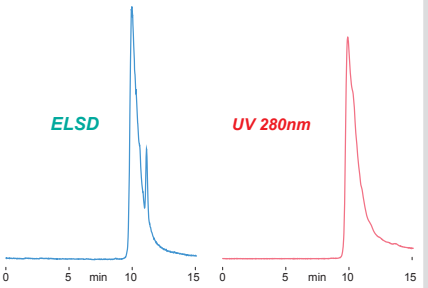
100mM ammonium formate 872

α-Amylase



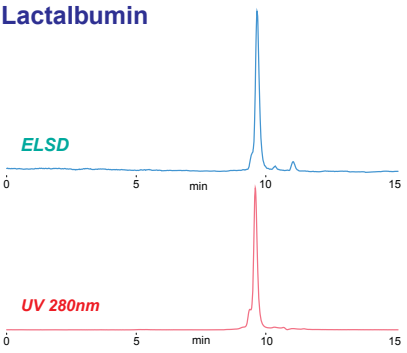
100mM ammonium formate 889

Hemoglobin



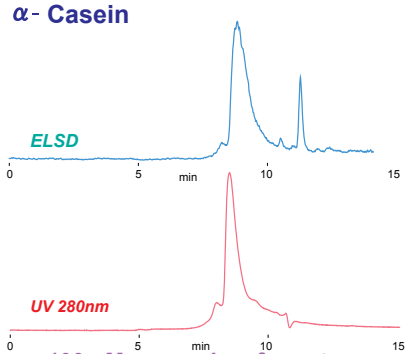
100mM ammonium formate 870

Lactalbumin



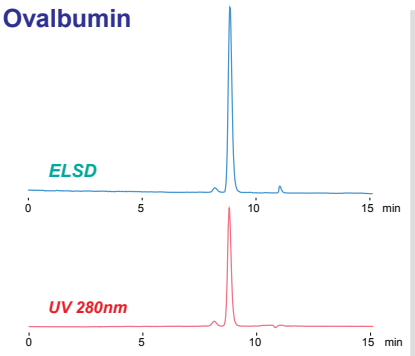
100mM ammonium formate 895

α-Casein



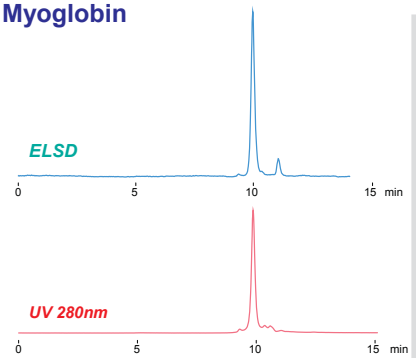
100mM ammonium formate 894

Ovalbumin



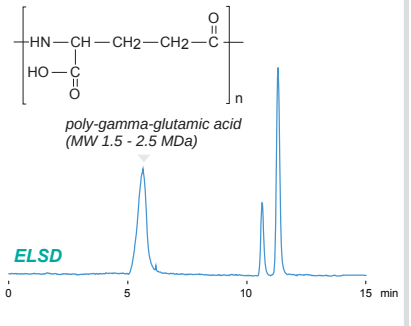
100mM ammonium formate 899

Myoglobin



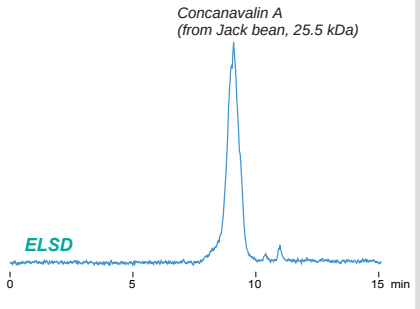
100mM ammonium formate 898

Polyglutamic acid



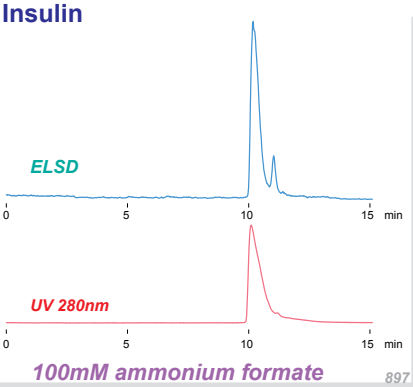
100mM ammonium formate 901

Lectin (Concanavalin A)



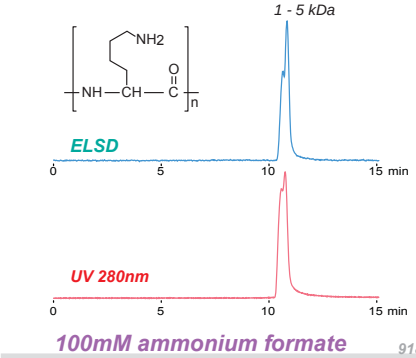
100mM ammonium formate 916

Insulin



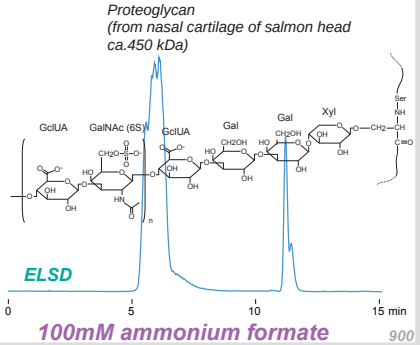
100mM ammonium formate 897

Poly-L-Lysine



100mM ammonium formate 918

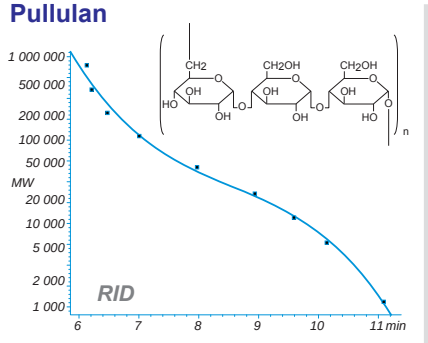
Proteoglycan



100mM ammonium formate 900

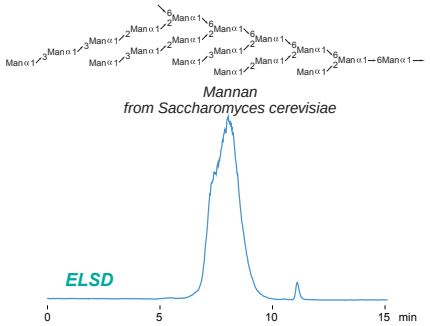
Intrada SEC, 250x4.6mm, 0.3mL/min, UV or ELSD

Pullulan



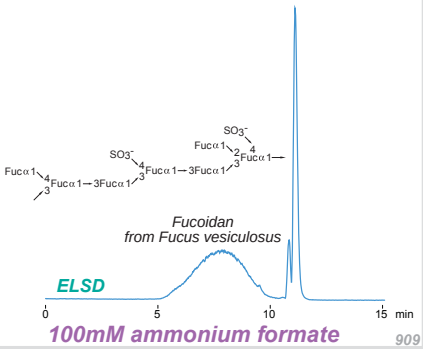
100mM ammonium formate 890

Mannan



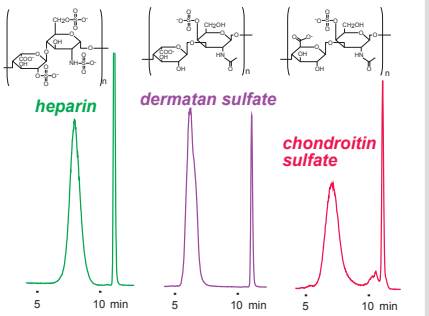
100mM ammonium formate 910

Fucoidan



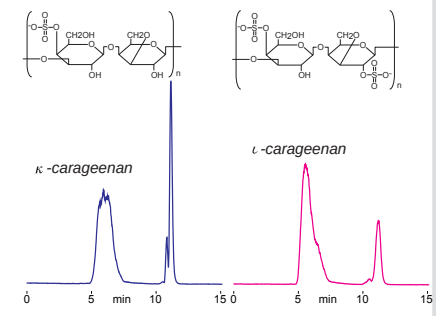
100mM ammonium formate 909

Glycosaminoglycan (GAGs) ELSD



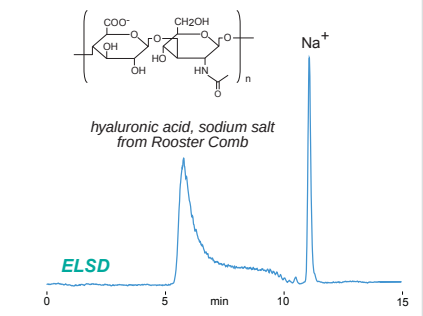
100mM ammonium formate 866

Carrageenan ELSD



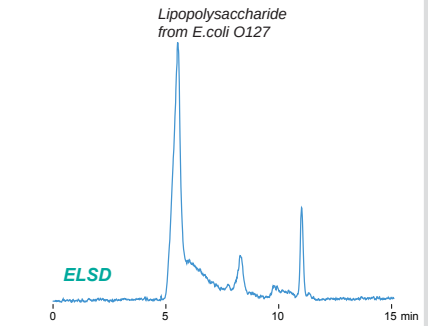
100mM ammonium formate 907

Hyaluronic acid



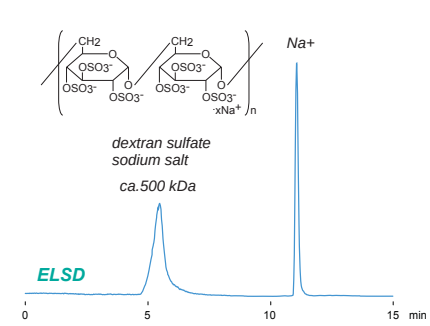
100mM ammonium formate 908

Lipopolysaccharide (LPS)



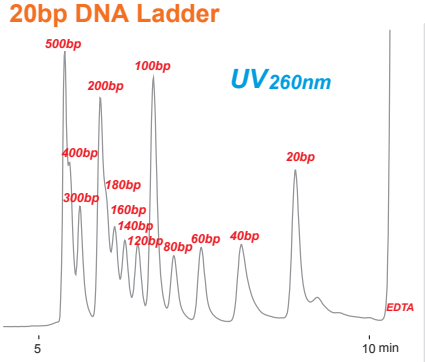
100mM ammonium formate 917

Dextran sulfate



100mM ammonium formate 902

20bp DNA Ladder

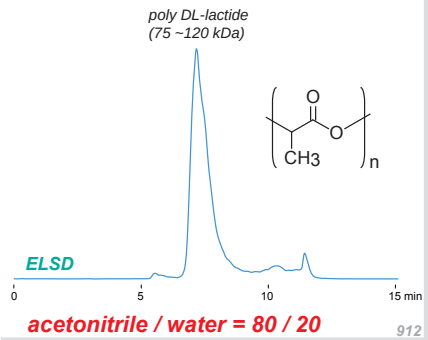


100mM ammonium formate 867

Synthetic polymers

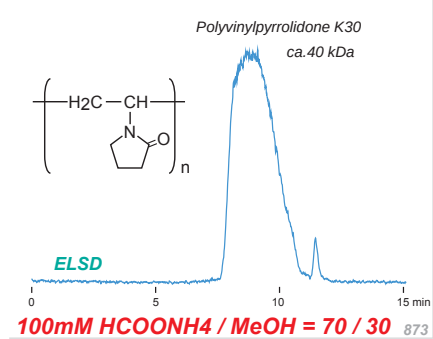
Synthetic polymers have a wide range of polarities and solubilities, so it is necessary to optimize both the sample solvent as well as the eluent, independently. Sample solvent should have a high solubility for the polymer, and the eluent should have a higher polarity than the sample, to ensure adequate elution.

Polylactide (PLA)



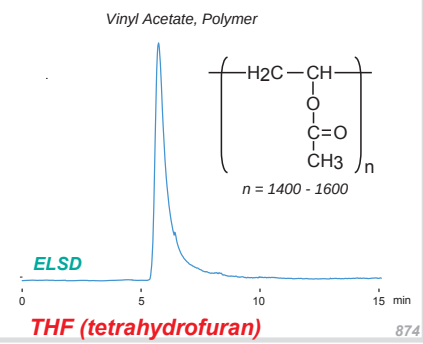
acetonitrile / water = 80 / 20 912

Polyvinylpyrrolidone (PVP)



100mM HCOONH4 / MeOH = 70 / 30 873

Polyvinyl acetate (PVAc)



THF (tetrahydrofuran) 874

Intrada SEC, 250x4.6mm, 0.3mL/min, UV or ELSD

Imtakt

Imtakt USA (info@imtaktusa.com)
North America

Imtakt Corp./JAPAN (info@imtakt.com)
Other Countries

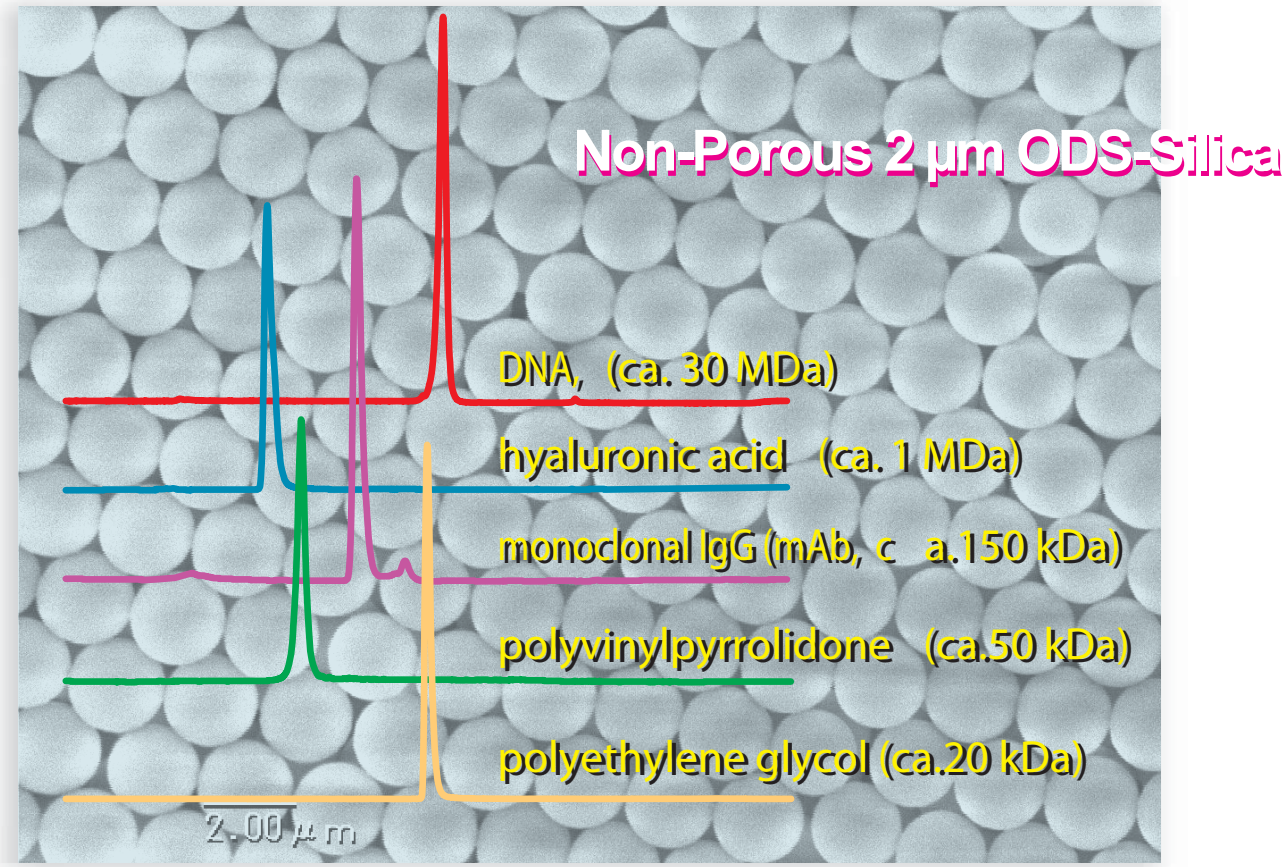
Presto FF-C18

High Resolution 2 µm Non-Porous ODS Column

Reversed-phase separation for bio- and synthetic-polymers up to 30 MDa
Amazing number of peaks for peptides and proteins
Different selectivity from porous ODS columns
250mm length high-resolution column with 2um particle
High-efficiency with low flow rate compatible with conventional HPLC systems

Non-Porous Spherical Silica / 2um / ODS

Changing polymer separation history



Imtakt has developed a novel 2um non-porous high resolution ODS column.

There are several shortcomings for porous ODS columns for polymer separation:

- Poor peak shape of solutes due to wide range in pore size distribution
- Poor recovery of solutes due to micro-pores and meso-pores
- Reduced column efficiency due to high mass transfer resistance

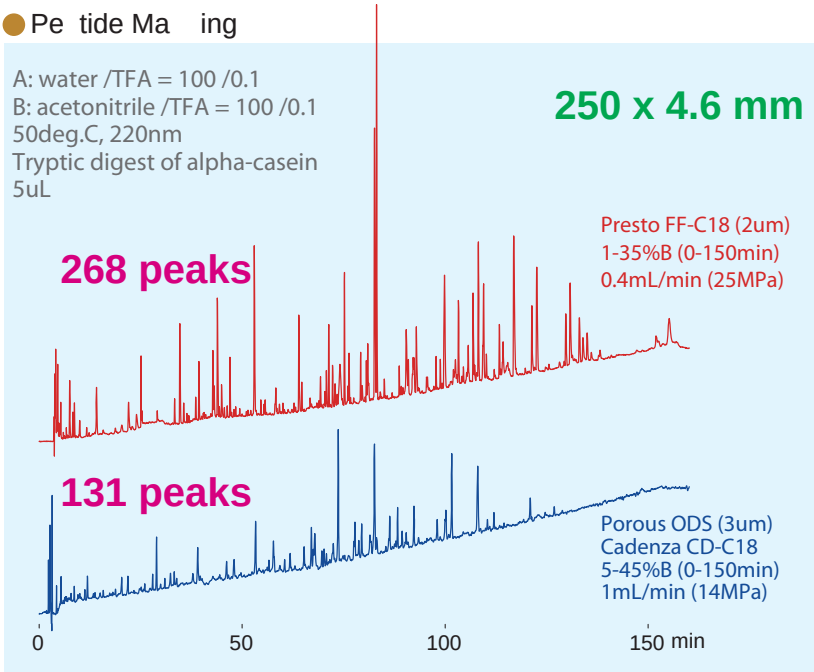
Presto FF-C18 can overcome these shortcomings for polymer separation. This high resolution, non-porous ODS column is quite different from conventional ODS columns, and will create new opportunities for 21st century separation science.

info@imtaktusa.com
www.imtaktusa.com
(888) 456-HPLC

Peptides

Presto FF- C18 performs exceptionally well with amino acid residue recognition for peptides and protein separations. The number of peaks for Presto FF- C18 is twice that of porous ODS - and shows improved recovery for peptides and proteins due to lack of micro- pores. The 250mm length Presto FF- C18 (2um particle) column will be an important tool for high resolution peptide and protein separations.

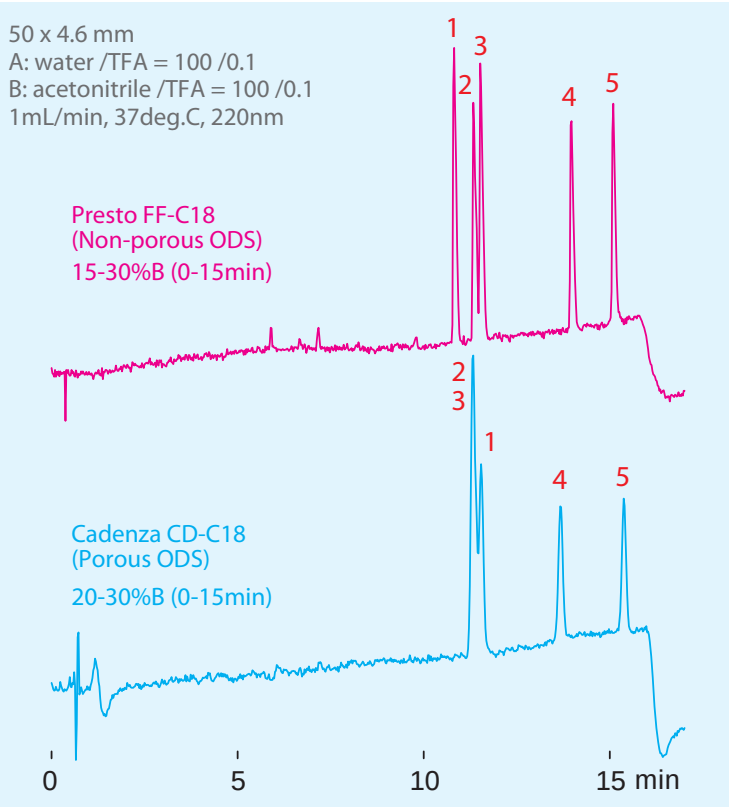
Pe tide Ma ing



Presto FF-C18 enables unbelievable separation power for peptide mapping. Accurate trace analysis for peptides is very important for proteome analysis, but can be compromised with porous ODS column - due to adsorption in micro-pores.

Presto FF-C18 offers twice the number of peaks and improved recovery over porous ODS columns, and will change the proteomics world dramatically.

Different electi it for Pe tide e aration



1 Angioten in I (uman)
Val-Tyr-Ile-His-Pro-Phe

2 Angioten in III (uman)
Arg-Val-Tyr-Ile-His-Pro-Phe

3 Angioten in II (uman)
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

4 al^5 -Angioten in I (Bo ine)
Asp-Arg-Val-Tyr-Val-His-Pro-Phe-His-Ile

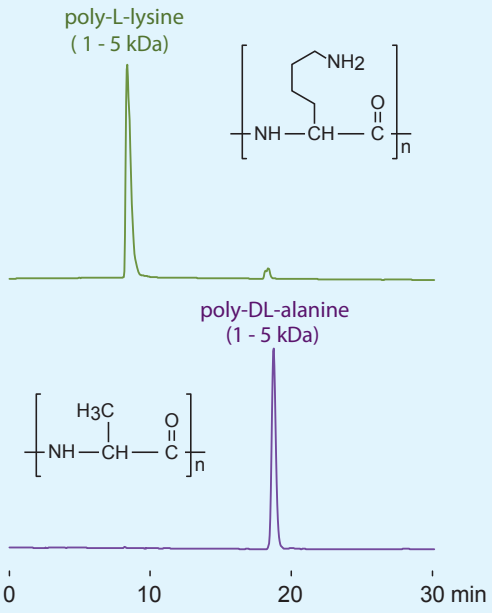
5 Angioten in I (uman)
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Ile

The surface area for non-porous ODS is extremely low compared to porous ODS. In order to obtain similar retention as porous ODS, the organic composition should be decreased when using non-porous ODS. This can be advantageous as the difference in eluent composition can contribute to different elution profile (as can be seen with the separation of angiotensin I, II and III).

Polypeptides and Proteins

Presto FF- C18 excels at (different) peptide bond structure recognition. It is useful for a wide range of molecular weight separations - from small peptides to large proteins.

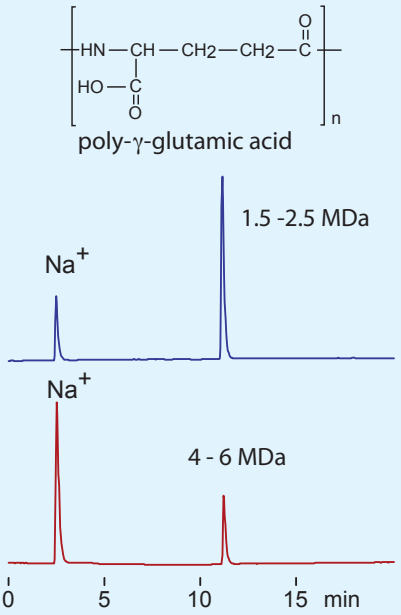
Polyamino Acid



Presto FF-C18, 250 x 4.6 mm
A: water / trifluoroacetic acid = 100 / 0.1
B: acetonitrile / trifluoroacetic acid = 100 / 0.1
0-60 % B (0-20min)
0.2 mL/min (13MPa), 37 deg.C
ELSD, 2-3uL (10-12ug)

580

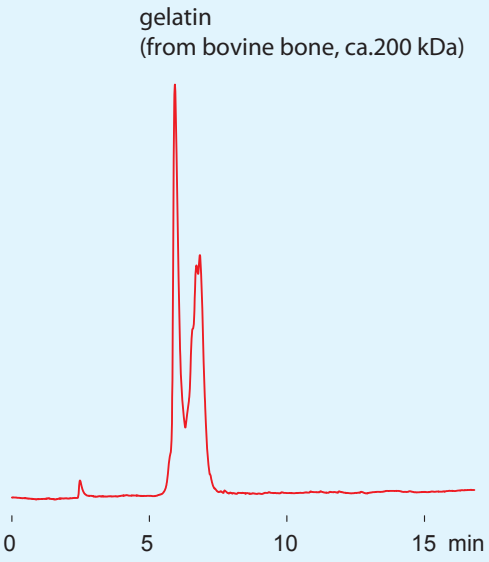
Polyglutamic Acid



Presto FF-C18, 150 x 4.6 mm
A: water / formic acid = 100 / 0.1
B: acetonitrile / formic acid = 100 / 0.1
0-30% B (0-15min)
0.4 mL/min (16MPa), 37 deg.C
ELSD, 2 uL (10ug)

560

Gelatin



Presto FF-C18, 150 x 4.6mm
A: water / trifluoroacetic acid = 100 / 0.1
B: acetonitrile / trifluoroacetic acid = 100 / 0.1
10-90 % B (0-15min)
0.4 mL/min (19MPa), 37 deg.C
ELSD, 2 uL (10ug)

567

Polyamino acids are polymers made up of repeating units of amino acids (shown are homo polyamino acids for L-lysine and DL-alanine). The two homopolymers provide very different retention times. Although the polyamino acids have a molecular weight distribution, the data shows only one peak for each polymer.

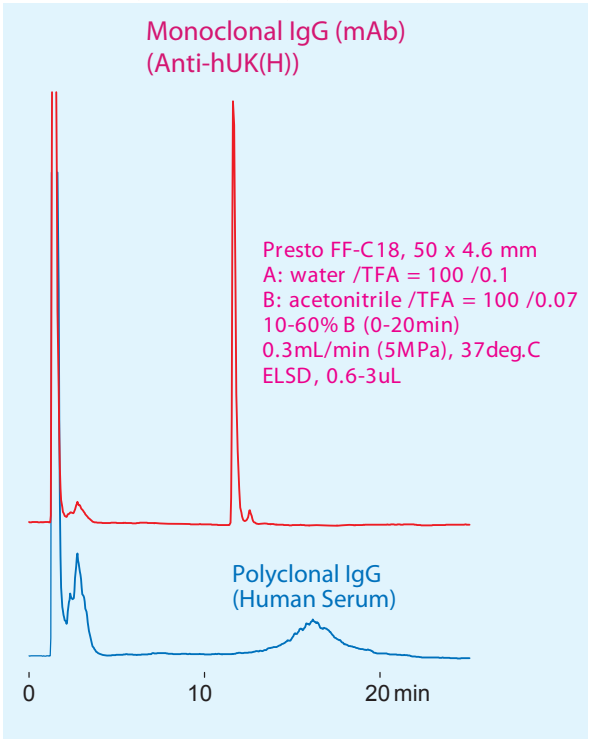
Polyglutamic acid (MW equals several MDa) has a wide range molecular weight distribution. However, polymers of similar molecular weight co-elute to form one peak. The reason for the poor recognition of chain length is as follows: the size of the polypeptide has reached a critical mass where the contact area to stationary phase is effectively the same for all polymers. The molecular interaction between solute and stationary phase is very similar, regardless of molecular weight of polymers - making it difficult to differentiate between the structures.

In contrast, proteins consist of a multitude of different amino acid residues. As a result, they are well differentiated on Presto FF-C18 (regardless of the molecular weight). Gelatin consists of molecular weight distribution - and can be separated by Presto FF-C18.

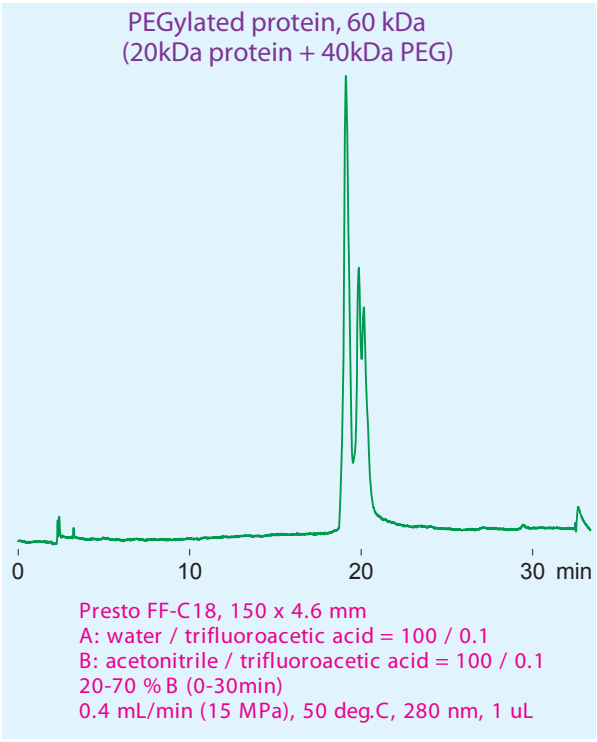
Medical Related Proteins

Presto FF- C18 also offers excellent results for medical related proteins. The more traditional modes of separation for proteins, IEX or SEC, can be replaced with reversed- phase using non- porous ODS.

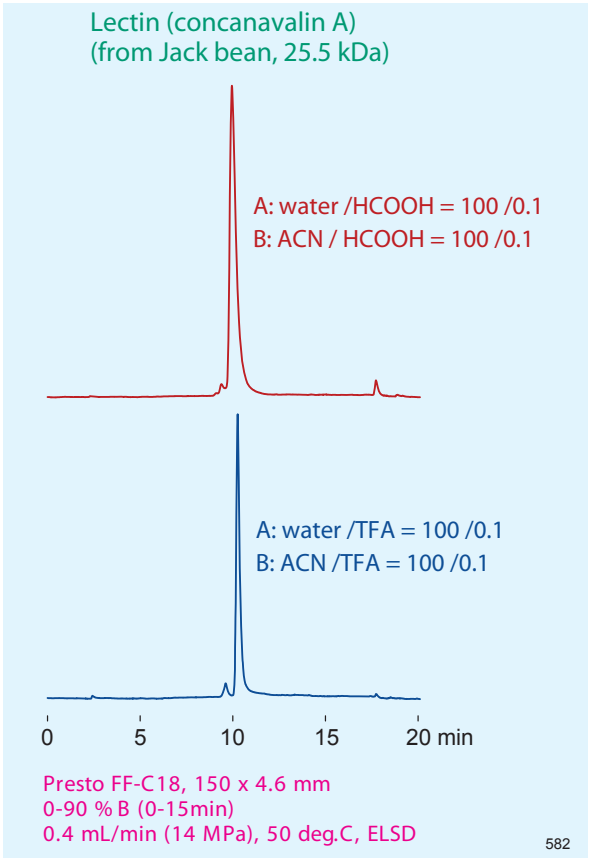
Immunoglobulin G (IgG) antibody



PEGylated Protein



Lectin



The Immunoglobulin G (IgG,) antibody, which consists of a large quaternary structure (MW ca. 150kDa), can exhibit poor peak shape when injected onto porous RP columns (due to pore size distribution and mass transfer resistance). Presto FF-C18 can provide improved peak shape and recognition between mAb (same amino acid sequence) and polyclonal antibody (different amino acid sequence).

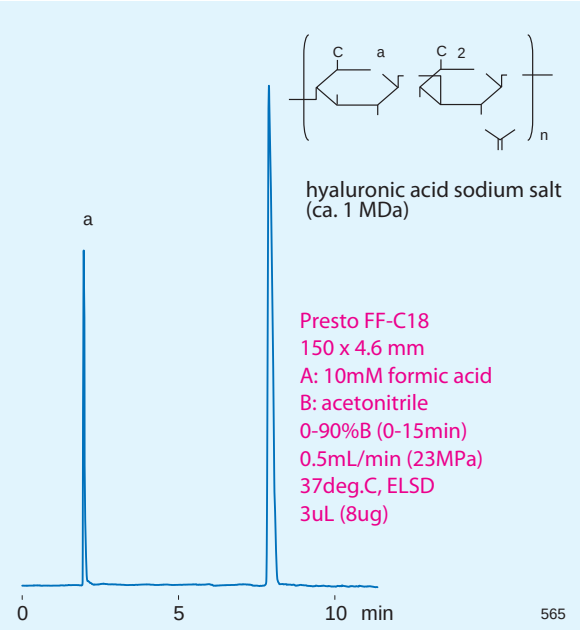
The PEGylation of proteins offers many advantages for drug and protein therapeutics. The above data shows several peaks due to various PEG oligomers and / or different yields of PEGylation. Presto FF-C18 will be useful for purity check, structural analysis, and QC production regarding almost any protein.

Lectin, a protein which binds to specific sugars, is important for medical purposes. The Concanavalin A protein elutes easily using formic acid / acetonitrile gradient.

Polysaccharides (Ionic)

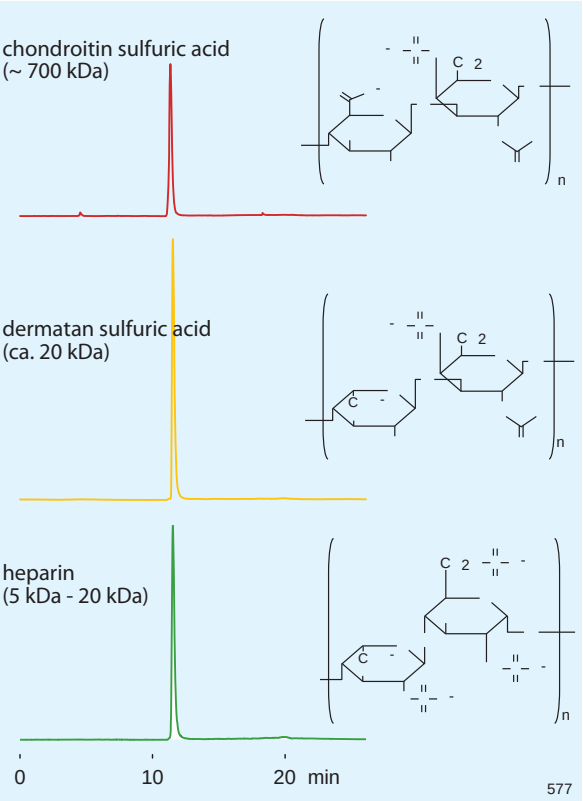
Presto FF-C18 (non-porous ODS) can be useful for polysaccharide separations. In comparison to SEC columns, Presto FF-C18 can achieve sharper peaks using gradient elution, making RP the preferred mode for quantitative analysis.

aluronic Acid

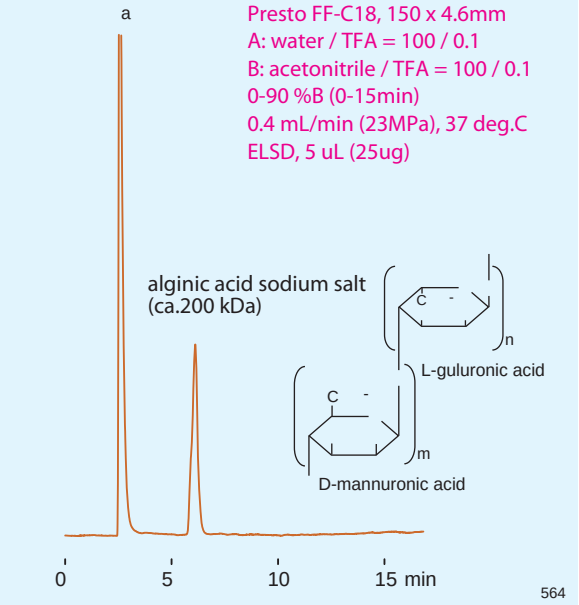


Presto FF-C18, 250 x 4.6 mm
A: water /triethylamine /acetic acid = 100 /1.1 /0.5
B: acetonitrile
0-40% B (0-15min)
0.35 mL/min (24 MPa), 37 deg.C, ELSD, 2 uL (10 ug)

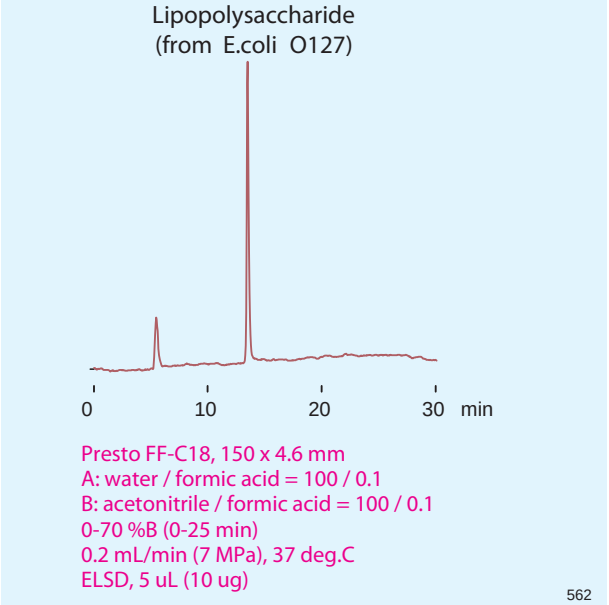
Muco ol acc aride



Alginic Acid



Li o ol acc aride



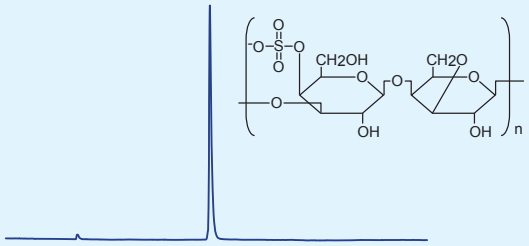
pH modifiers are required when ionic polysaccharides are injected on to Presto FF-C18. Hyaluronic acid, a mucopolysaccharide, elutes with formic acid (even though it contains a carboxyl group). In contrast, chondroitin, dermatan, and heparin contain sulfur group(s) and require triethylamine acetate (ion-pairing modifier) under neutral pH conditions. Retention behavior for these three polymers is similar due to similar structure and molecular weight. Trifluoroacetic acid (TFA), which was used for alginic acid, can also be useful for ionic polysaccharide separations.

Polysaccharides (Ionic)

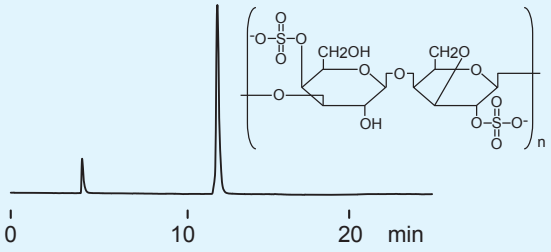
Presto FF- C18 is useful for polysaccharide analysis.

Carrageenan

kappa-carrageenan
(~ 1 MDa)



iota-carrageenan
(~ 1 MDa)

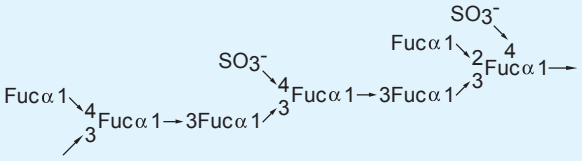


Presto FF-C18, 250 x 4.6 mm
A: water /triethylamine /acetic acid = 100 /1.1 /0.5
B: acetonitrile
0-40 %B (0-15min)
0.35 mL/min (24 MPa), 37 deg.C
ELSD, 2 uL (10 ug)

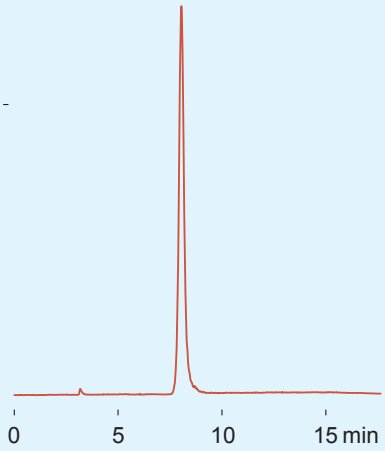
563

Courtesy of Anthony Montanari, Ph.D., Perrigo Company of South Carolina - USA

Fucoidan



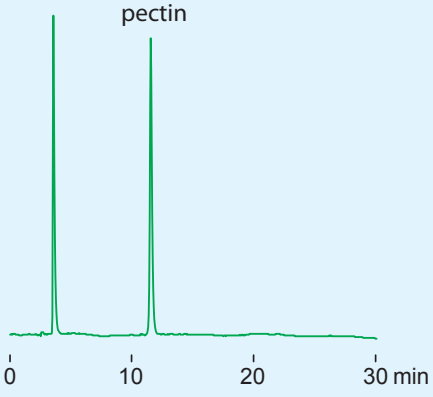
fucoidan
(from Fucus vesiculosus , 100 - 180 kDa)



Presto FF-C18, 150 x 4.6 mm
A: water /triethylamine /acetic acid = 100 /1.1 /0.5
B: acetonitrile
0-70 %B (0-15min)
0.3 mL/min (14 MPa), 37 deg.C
ELSD, 2 uL (10 ug)

584

Pectin



Presto FF-C18, 150 x 4.6 mm
A: water / formic acid = 100 / 0.1
B: acetonitrile / formic acid = 100 / 0.1
0-50 %B (0-25 min)
0.3 mL/min (11 MPa), 37 deg.C
ELSD, 2 uL (4 ug)

561

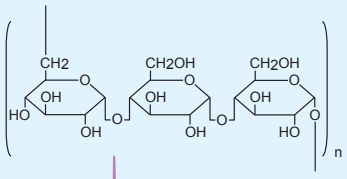
A pH modifier is required to analyze ionic polysaccharides. Triethylamine acetate in particular is effective for polysaccharides that contain sulfur groups. The two carrageenan (κ-,ι-) shown here have a different number of sulfur groups. However, retention (and peak shape) is similar for both polysaccharides due to their similar structures and molecular weights. Fucoidan contains sulfur groups and results in excellent peak shape when triethylamine acetate pH modifier is used.

Pectin is comprised of repeating units of galacturonic acid. Although a majority of these units are esterified - a percentage of the units are not. Formic acid was found to be a useful pH modifier for this application.

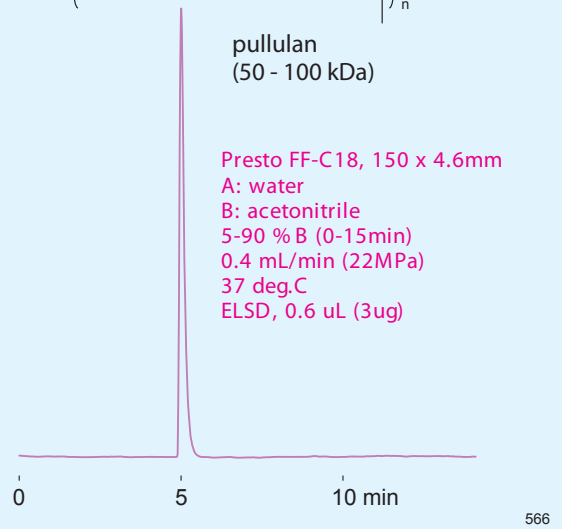
Non-Ionic Polysaccharides

Presto FF- C18 separates polysaccharides and does so with sharper peaks and lower cost than SEC columns.

Pullulan

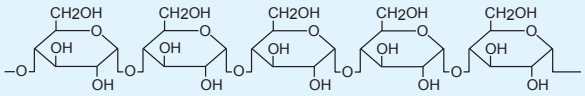


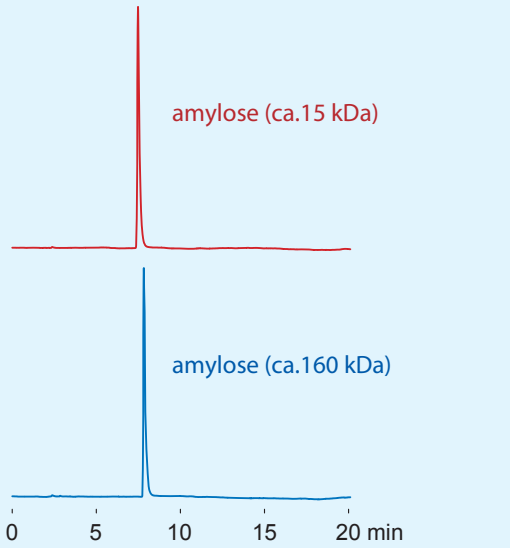
pullulan
(50 - 100 kDa)



566

Amylose

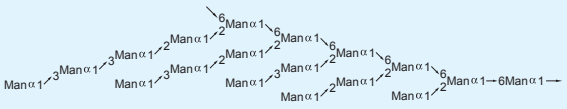




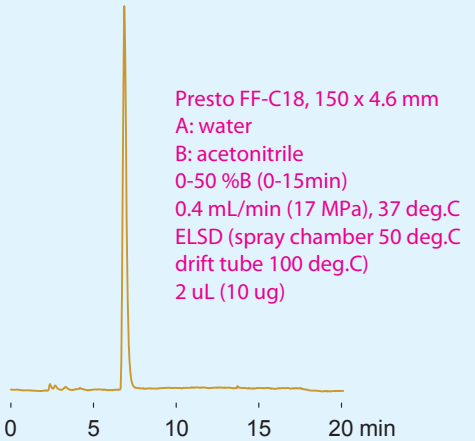
Presto FF-C18, 150 x 4.6 mm
A: water, B: acetonitrile
0-50 % B (0-15min), 0.4 mL/min (17 MPa), 37 deg.C
ELSD, 1 uL (5 ug, 0.5N-NaOH)

585

Mannan

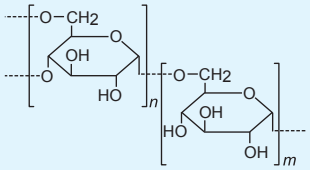


mannan
(from Saccharomyces cerevisiae ,
ca.130 kDa)

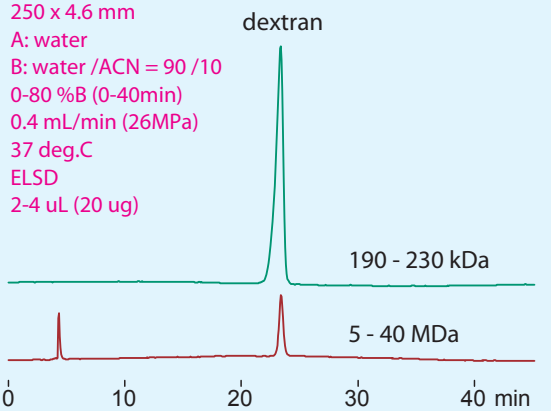


583

Dextran



Presto FF-C18
250 x 4.6 mm
A: water
B: water /ACN = 90 /10
0-80 %B (0-40min)
0.4 mL/min (26MPa)
37 deg.C
ELSD
2-4 uL (20 ug)



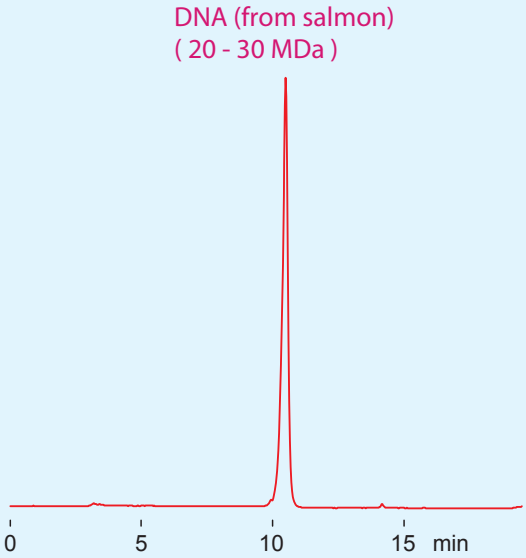
579

Reversed-phase separation for non-ionic polysaccharides on Presto FF-C18 does not require any pH modifier. Excellent peak shape was observed for several non-ionic polysaccharides using water / acetonitrile gradients. Polysaccharides contain multiple OH groups, and different concentrations of acetonitrile may be required for proper elution. The data shows that retention will be similar for homo polysaccharides larger than 10kDa. Peak shape is dependent upon molecular size distribution.

Nucleic Acids

Presto FF-C18 can analyze nucleic acids. Historically, nucleic acids have been difficult to analyze by HPLC, due to the extremely large size of these biopolymers. The non-porous ODS, Presto FF-C18, makes this possible.

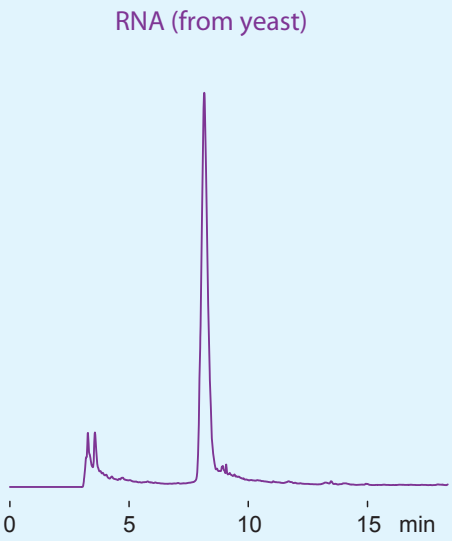
DNA



Presto FF-C18, 150 x 4.6 mm
A: 50 mM ammonium acetate
B: acetonitrile
0-50 % B (0-15min)
0.3 mL/min (12MPa), 37 deg.C, 260 nm
1 uL (4ug, 50mM ammonium acetate)

557

RNA



Presto FF-C18, 150 x 4.6 mm
A: 50 mM ammonium acetate
B: acetonitrile
0 - 60 % B (0-20 min)
0.3 mL/min (11MPa)
50 deg.C, 260 nm, 0.6uL (1.5 ug)

587

Plasmid DNA

Presto FF-C18, 250 x 4.6 mm
A: 200 mM ammonium acetate
B: acetonitrile
0-30 % B (0-20min)
0.3 mL/min (17MPa), 50 deg.C, 260 nm, 1 uL (4ug)

558

Double stranded DNA is a tremendously large molecule that is normally hydrolyzed into fragments (via nuclease) prior to analysis. Presto FF-C18 can analyze intact DNA as well as DNA fragments.

Increasing column temperature to 50 deg.C can help to improve peak shape for both DNA and RNA. In addition, 50mM ammonium acetate, used as a neutral pH modifier, can also help to improve peak shape.

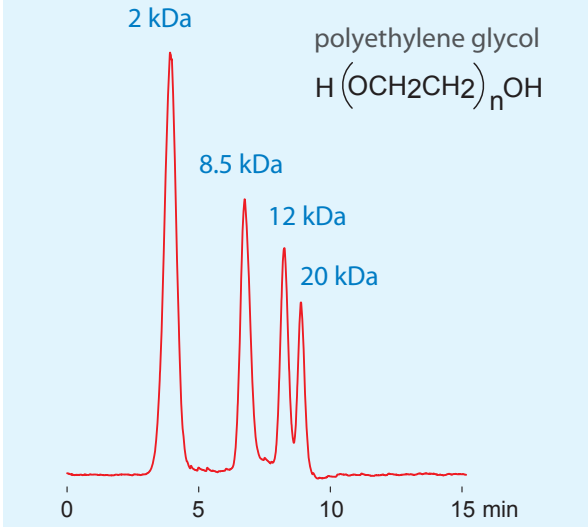
Plasmid DNA consists of covalently closed circular form and has a different structure from nuclear DNA. In this experiment, a high concentration of ammonium acetate (200mM) was required for elution.

Porous RP columns traditionally have struggled with DNA and RNA analysis. Presto FF-C18 can improve both analysis and isolation of these large biopolymers.

Synthetic Polymers (Hydrophilic)

Presto FF- C18 can achieve better peak shape for synthetic polymers, which have traditionally been analyzed on SEC mode. In addition, peak width under RP mode is dependent upon molecular size distribution.

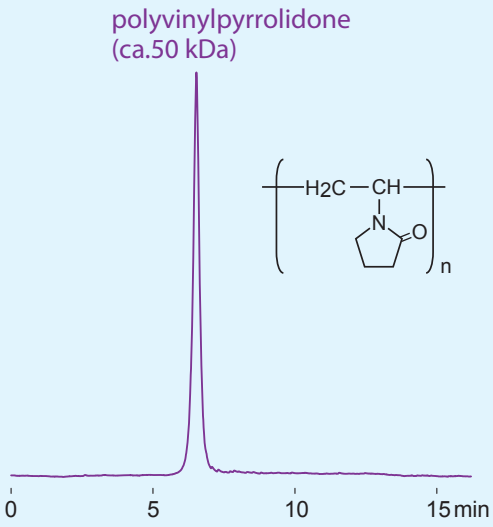
Polyethylene Glycol (PEG)



Presto FF-C18, 150 x 4.6mm
A: water
B: acetonitrile
30-40 % B (0-2min), 40-60 % B (2-15min)
0.4 mL/min (24MPa), 37 deg.C
ELSD, 3 uL (1.5-6ug)

571

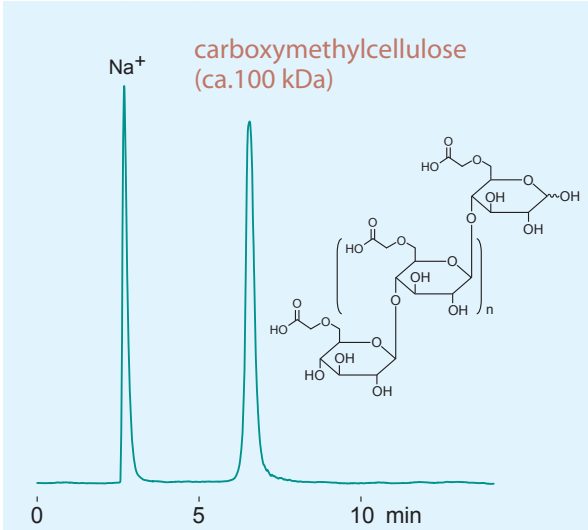
Polyvinylpyrrolidone (PVP)



Presto FF-C18, 150 x 4.6mm
A: water /trifluoroacetic acid = 100 /0.1
B: acetonitrile /trifluoroacetic acid = 100 /0.1
20-60 % B (0-10min)
0.4 mL/min (23MPa), 37 deg.C
ELSD, 1 uL (5ug)

570

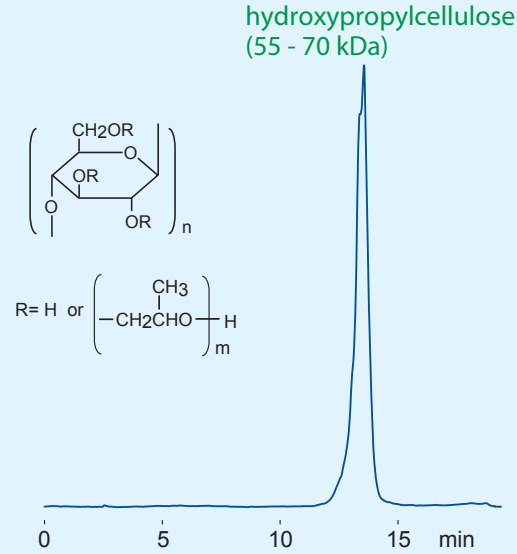
Carboxymethylcellulose (CMC)



Presto FF-C18, 150 x 4.6mm
A: water / trifluoroacetic acid = 100 / 0.1
B: acetonitrile / trifluoroacetic acid = 100 / 0.1
5-60 % B (0-10min),
0.4 mL/min (23MPa), 37 deg.C
ELSD, 8 uL (16ug)

566

Hydroxypropylcellulose (HPC)



Presto FF-C18, 150 x 4.6mm
A: water
B: acetonitrile
5-90 % B (0-15min)
0.4 mL/min (21MPa), 37 deg.C
ELSD, 2 uL (10ug)

569

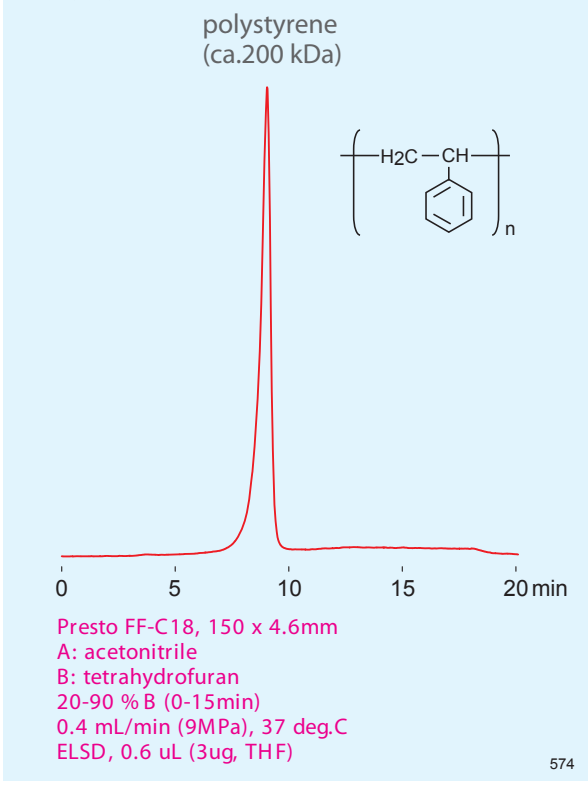
Hydrophilic polymers, which have been traditionally analyzed on aqueous SEC (GFC) mode, may now also be analyzed on Presto FF-C18 with RP mode and gradient elution.

The PEG data shows that relatively small polymers (up to 100kDa) can be separated from each other. Ionic polymers, such as CMC, require the addition of pH modifier to the eluent.

Synthetic Polymers (Hydrophobic)

Presto FF-C18 can be applied to hydrophobic polymer analysis. The solubility and elution properties of the solute need to be taken into account when preparing the mobile phase. There is an opportunity to reduce costs and convert the current GPC method to an RP method with Presto FF-C18.

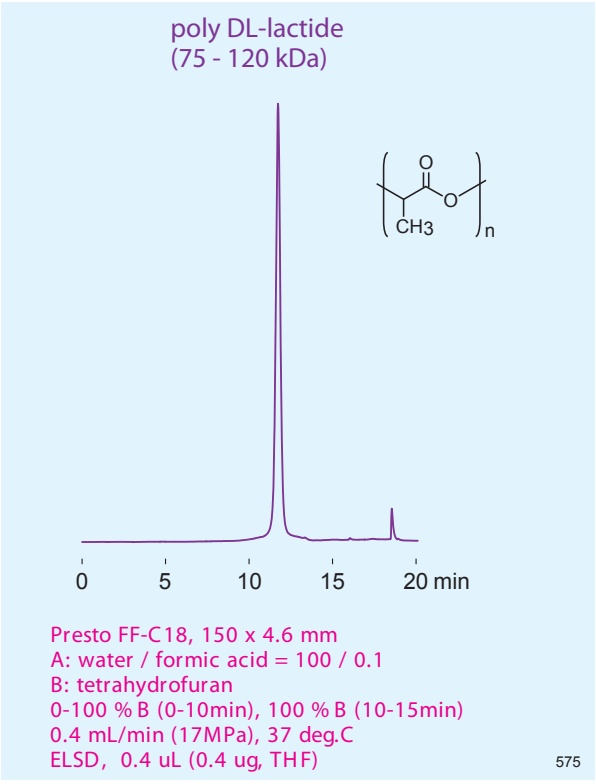
Polystyrene



Presto FF-C18, 150 x 4.6mm
A: acetonitrile
B: tetrahydrofuran
20-90 % B (0-15min)
0.4 mL/min (9MPa), 37 deg.C
ELSD, 0.6 uL (3ug, THF)

574

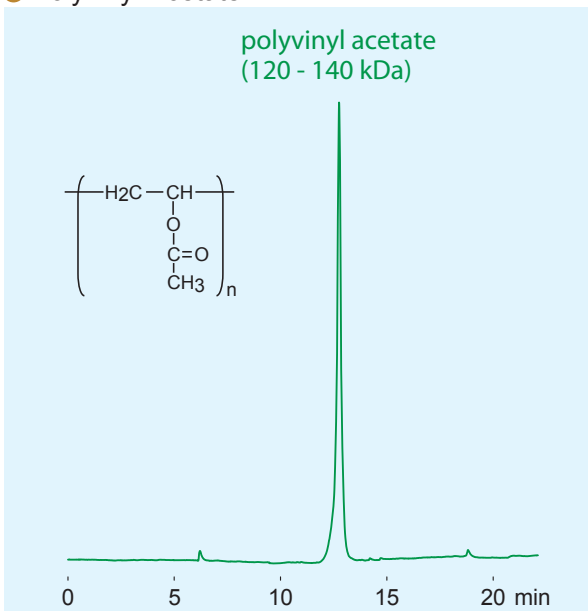
Polylactide



Presto FF-C18, 150 x 4.6 mm
A: water / formic acid = 100 / 0.1
B: tetrahydrofuran
0-100 % B (0-10min), 100 % B (10-15min)
0.4 mL/min (17MPa), 37 deg.C
ELSD, 0.4 uL (0.4 ug, THF)

575

Polyvinyl Acetate



Presto FF-C18, 150 x 4.6 mm
A: water
B: acetonitrile
0-100 % B (0-10min), 100 % B (10-15min)
0.4 mL/min (15MPa), 50 deg.C,
ELSD, 1 uL (5 ug, methanol)

573

Polystyrene, a large molecule, is very hydrophobic and difficult to analyze on porous RP columns. Usually it is analyzed using GPC. Presto FF-C18 can be used with non-aqueous elution. Peak width will be affected by molecular size distribution.

Polylactide is hydrophobic polymer, but does have some hydrophilic properties due to its large abundance of oxygen. As a result, gradient elution from water to THF was required.

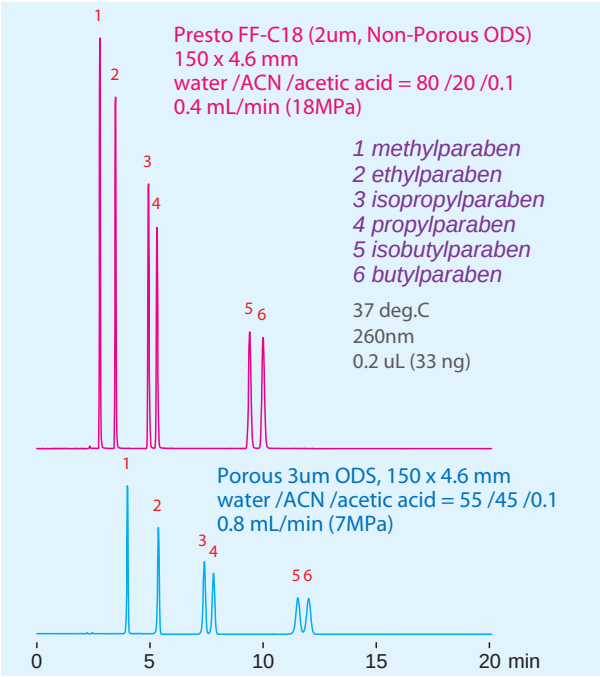
Similarly, polyvinyl acetate is a hydrophobic polymer with some hydrophilic properties. It required gradient elution from water to acetonitrile.

Presto FF-C18 can create new possibilities for hydrophobic polymer separations under RP mode.

all o o nds

Due to its extremely low surface area (non-porous), Presto FF-C18 is usually not recommended for small molecule analysis. However, depending on the solute structure, non-porous ODS can be advantageous for small molecule separations.

Low Flow Rate - ig en iti it and Ama ing e aration

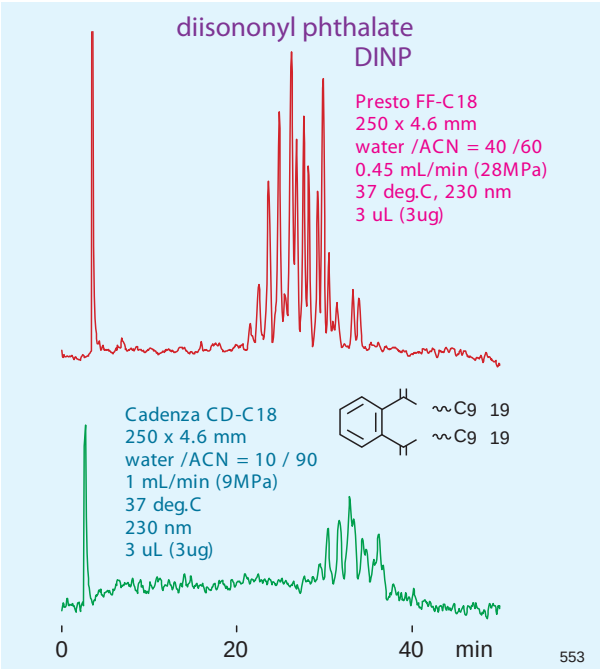


When mobile phase composition and flow rate is optimized, Presto FF-C18 can at times achieve amazing peak shape for small molecules.

Porous ODS has a high mass transfer resistance (resulting in band broadening). In contrast, non-porous ODS does not have this issue. As a result, for the alkylparabens, Presto FF-C18 provided better peak shape (less band broadening) than porous ODS.
In addition, lower flow rate (ex: half the flow rate of porous ODS column) provides improved sensitivity and resolution.

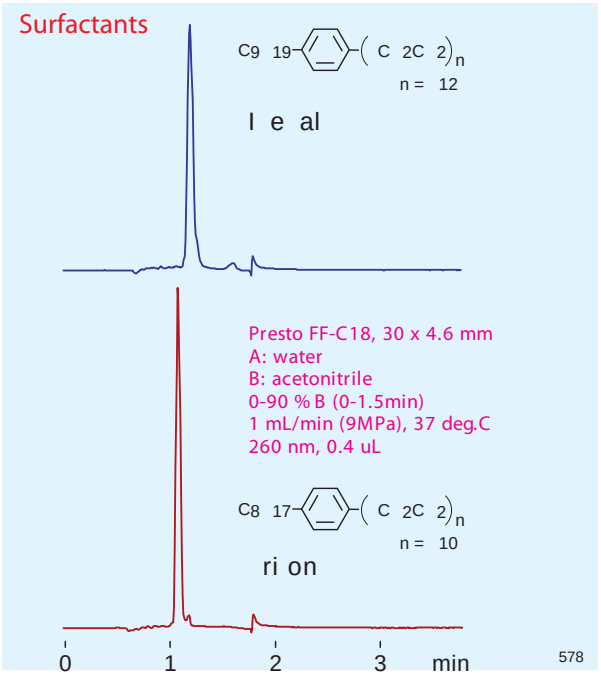
Although Presto FF-C18 consists of 2um particles, there are many advantages to operating at low flow rate: higher resolution, improved sensitivity, and lower pressures for conventional HPLC systems.

Recogni e Al l Branc ed-C ain



On conventional porous ODS, Phthalic di-ester has numerous branched-chains with the same MW that co-elute. In contrast, Presto FF-C18, at a low flow rate, recognizes the alkyl chain isomers and is more sensitive.

Pea Co- lute to Ma e ne Pea



With small compounds, Presto FF-C18 sometimes shows worse separation than porous ODS columns. Igepal and Triton are mixtures of different oxyethylene chain lengths. Porous ODS column can easily separate the oligomers, while Presto FF-C18 provides poor selectivity due to low oxygen recognition. However, having the oligomers co-elute on Presto FF-C18 can be favorable since it produces one peak in a short time analysis.

Presto FF-C18 can expand separation possibilities when coupled with porous ODS columns.

eco enda ions or Pres o

Pre to FF-C18 i a non- orou D column con i ting of 2um non- orou ilica article . T e ecific urface area i muc lower t an con entional orou D column. A a re ult, eluent com o ition ould be o timi ed in order to obtain retention e ui alent to a orou D column. Pre to FF-C18 wor great at low flow rate and can be u ed on con entional PLC tem . ig er flow rate (i.e. ame flow rate a orou D column) will re uire t e u e of a PLC tem.

o ile Phase o osi ion

Retention is greatly reduced on non-porous ODS due to its extremely low surface area. In order to get similar retention to porous ODS, organic solvent ratio should be reduced by 1/2 - 1/3. But this leads to another benefit as it decreases solvent consumption.

lo a e

Presto FF-C18 consists of 2um particles and can be used at operating pressures up to 50MPa for 6mm or less I.D. columns. In addition, because Presto FF-C18 is non-porous (and thus no diffusion in pores), excellent performance is also achieved at lower flow rates. This has the added effect of being able to use 2um particle on conventional HPLC systems. Additionally, lower flow rates, such as 1/2 - 1/3 of porous ODS columns, improves resolution and sensitivity.

a le ol en and In ec ion ol e

The surface area of Presto FF-C18 is extremely low and can be affected by sample injection volume. In addition, peak shape may be poor when polar compounds are dissolved in organic solvent and operated under highly aqueous eluent conditions. Under this scenario, the organic solvent becomes the eluent - causing some solutes to elute more quickly than others (resulting in poor peak shape). To avoid this potential problem, sample solvent should be highly aqueous (which also allows the use of larger injection volumes).

e en ion o Polar o o nds

Presto FF-C18 can struggle to retain polar compounds. But, 100% aqueous eluent can be used with this column and analysis of polar compounds can be optimized by using 0.1-1% organic.

l ion ode

Presto FF-C18 has a disadvantage for isocratic elution due to low surface area. It is therefore recommended to use gradient elution as often as possible. If isocratic elution is required, peak shape and retention reproducibility can be improved by increasing the ionic strength of the buffer.

eco enda ions or P era ion

It is recommended that low dispersion systems and high pressure binary pumps are used with Presto FF-C18. Low pressure gradient pumps have too much dead volume which can have a negative effect on the separation. It is highly recommended to use gradient elution with Presto FF-C18. If isocratic elution is required, it is strongly recommended to pre-mix the mobile phase prior to use. Due to the non-porous silica, the kinetics within Presto FF-C18 are extremely fast. Therefore, a mixer is not required with this column (t-union is sufficient for HT analysis).

rderin In or a ion

Presto FF-C18 2 m on-Porou ilica, ctadec l Ligand, nd-Ca ed

2µm

Lengt (mm)	Product Code					
	1 mm I.D.	2 mm I.D.	3 mm I.D.	4.6 mm I.D.	6 mm I.D.	10 mm I.D.
10	-	FF020	FF030	FF000	-	-
20	-	FF029	FF039	FF009	-	-
30	FF011	FF021	FF031	FF001	FF061	FF0P1
50	FF012	FF022	FF032	FF002	FF062	FF0P2
75	FF013	FF023	FF033	FF003	FF063	FF0P3
100	FF014	FF024	FF034	FF004	FF064	FF0P4
150	FF015	FF025	FF035	FF005	FF065	FF0P5
250	FF016	FF026	FF036	FF006	FF066	FF0P6

Micro / ano column are al o a ailable.